ERRATUM

Article by H. A. Blair, 1932, Volume 29, Page 617, should read

$$p = \frac{KV_0}{\sqrt{k^2 + w^2}}$$

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6031

Local Formation of Antibody by the Nasal Mucosa.

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The importance of the upper respiratory tract as a portal of entry for pathogenic micro-organisms amply justifies attempts to increase its resistance against their invasion. We have recently shown[1] that the local introduction of antigen into an area of mobilized histiocytes leads to the local formation of specific antibody. We have extended such studies to the nasal mucosa, assuming that a similar response might be obtained there following repeated applications of an antigen. This assumption was based on the probable mobilization of cells of inflammation in the mucosa, with consequent fixation of antigen among differentiated histiocytes.

Rabbits were treated intranasally at daily intervals of 2 to 13 days

[1] Cannon, Paul R., and Sullivan, F. L., PROC. SOC. EXP. BIOL. AND MED., 1932, 29, 517.

with a formolized vaccine of *Bact. paratyphosum B.*, either by insufflation (2), by instillation alone (12), or by instillation subsequent to earlier instillation of ox bile (1). The animals were then allowed to rest for 1 to 12 days, and were sacrificed. The nasal mucosa, lung, liver, spleen, and blood serum were mixed with 15 parts of a solution of equal parts of glycerol and 0.85% solution of sodium chloride. They were then ground in a mortar, with the exception of the serum, and extracted at 37°C. for 7 days. These extracts were titrated simultaneously against a living suspension of *Bact. paratyphosum B.* Six of the 19 animals were perfused with citrated salt solution immediately after death to remove the blood as far as possible from the organs to be extracted. Most of the blood was removed from all except the spleen.

The content of agglutinin in the nasal mucosa in animals treated daily by insufflation or instillation for at least 11 days was always distinctly higher than that of either the spleen or liver. This was also true of the lung in all but one instance, when the titre equalled that of both spleen and liver. Untreated control animals when extracted and titrated under comparable conditions contained no agglutinin in the organs above mentioned. Six animals, treated 5 times a day for 2 days and killed from 24 to 96 hours after the last instillation, yielded no specific agglutinin at a dilution of 1:120 or above. In the animals with a high agglutinin content of the nasal mucosa and lung, the agglutinin content of the blood serum was higher than that of either the nasal mucosa or lung alone, with one exception when the titres of serum and nasal mucosa were the same.

We conclude, therefore, that the daily local insufflation or instillation of antigen for 11 days led to the local formation of specific agglutinins in the nasal mucosa and lung. Inasmuch as the agglutinin content of the liver and spleen was uniformly much lower and frequently absent entirely, while that of the blood serum was relatively high, we believe that the antibodies were formed *in loco* in the nasal mucosa and lungs and from there diffused into the blood.

It would seem, therefore, that if a local concentration of specific antibodies is a desirable condition in immunity, it may be obtained by the introduction locally, under suitable conditions, of the appropriate antigen. Such a reinforcement of a major portal of entry against bacterial infection should strengthen significantly the resistance of these tissues to the entrance of pathogenic micro-organisms. To that extent, the burden usually borne by the secondary general mechanisms of defence should be lessened. Further experiments along these lines are now in progress.

6032

Blood Cholesterol in Experimental Hypo- and Hyperthyroidism.*

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Changes in blood cholesterol in both clinical and experimental hypo- and hyperthyroidism have been reported recently by several workers. Briefly stated, hypothyroidism is accompanied by an increased blood cholesterol; hyperthyroidism by a decreased blood cholesterol.

This study was undertaken to confirm these findings in experimental hypo- and hyperthyroidism in rabbits, and to see if any correlation exists between cholesterol changes and changes in the number of red blood cells and Hb.

The technique for preparing cretin rabbits has been previously cited.[1] Blood counts were made in the usual way; Hb was determined colorimetrically[2] and is expressed in gm. per 100 cc. blood. Cholesterol was determined on whole blood by Sackett's[3] modification of Bloor's method. All determinations were done in duplicate.

Three cretin and 6 normal animals were used in the first series. These were fed from 0.318 to 0.59 gm. desiccated thyroids daily for 2 or 4 weeks. Thyroid feeding caused a decrease in the blood cholesterol of all rabbits of this series, but this decrease is not accompanied by a consistent change in the number of red blood cells or in Hb.

A second series was studied, using 2 cretins and 4 of the animals in series I. After thyroid feeding, the losses of weight and the decrease in blood cholesterol of the 2 cretins are almost identical. There is no corresponding change in red blood cells and Hb. In the 4 normal animals, the weight loss is again roughly uniform, but again, there is no corresponding change in cholesterol and in red blood cells or Hb.

The results of these experiments seem to indicate that the changes in blood cholesterol which follow thyroid feeding in both cretin and normal rabbits, show no correlation with changes in the number of red blood cells, or the amount of Hb.

* This investigation was aided by a grant from the Henry Strong Dennison Foundation, and from the Seymour Coman Fund.

[1] Kunde, M. M., *et al.*, *Am. J. Phys.*, 1927, **82**, 630.

[2] Newcomer, H. S., *J. Biol. Chem.*, 1923, **55**, 569.

[3] Sackett, G. E., *J. Biol. Chem.*, 1925, **64**, 203.

6033

Condensation Products of Lipoids and Chemo Immunity. I. Synthesis of Azo Derivatives of Cholesterol Aryl Esters.

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It has been proposed by Forssman,[1] Meyer,[2] Wernicke and Sordelli,[3] Taniguchi,[4] Landsteiner,[5] and Landsteiner and Simms[6] that some heterogenetic antigens may be composed of 2 chemically different substances, one being represented by a lipoidal substance containing the specific group but possessing no antigenic properties. The other component is of the nature of a protein. Since Landsteiner and Simms[6] reported investigations only with simple mixtures of these 2 systems, and Klopstock and Selter[7] those with mixtures of lecithin and diazotised atoxyl, it was of interest to study the possibility of chemically combining lipoidal substances with proteins and their degradation products to obtain a product of a definite entity which then could be used for immunological experiments. From a different aspect combination of lipoidal substances with proteins or their degradation products is interesting because of the colloidal nature of such condensations in an aqueous system. Such a physical state of the antigen is claimed to be important in immunity reactions.

The present report deals with experiments in which we prepared condensation products of cholesterol derivatives with certain amines, amino acids, and peptides. There are also indications of successful condensations with proteins.

Cholesterol, especially purified over the dibromide, was esterified with 4-nitrobenzoylchloride. The ester obtained was reduced with a Pt-catalyst as described by Shriner and Ko[8] to form the corresponding cholesteryl-4-aminobenzoate. For condensation with the above mentioned substances (amines, amino acids, peptides, etc.),

[1] Forssman, *Biochem. Z.*, 1911, **37**, 78.

[2] Meyer, *Z. f. Immunitatsf.*, 1911, **11**, 211; 1912, **15**, 245; 1913, **20**, 367; *Biochem. Z.*, 1921, **122**, 225.

[3] Wernicke and Sordelli, *Rev. Inst. bact. Buenos Aires*, 1919, **2**, 281.

[4] Taniguchi, *J. Path. Bact.*, 1921, **24**, 217.

[5] Landsteiner, K., *Biochem. Z.*, 1921, **119**, 294.

[6] Landsteiner and Simms, *J. Exp. Med.*, 1923, **38**, 127.

[7] Klopstock and Selter, *Z. f. Immunitatsf.*, 1928, **57**, 174.

[8] Shriner and Ko, *J. Biol. Chem.*, 1928, **80**, 1.

diazotisation of the amino benzoyl ester was studied. A number of difficulties were encountered. It was found, for example, that while the hydrochloride of the cholesteryl-4-aminobenzoate can also be prepared by carefully grinding it with an excess of conc. HCl, the resulting salt is not soluble in water.* The action of alkali nitrite therefore proved not to be effective, resulting in an incomplete diazotisation.

Finally, diazotisation with amylnitrite in chloroform, ether, butyl alcohol, etc., was investigated and chloroform found best suitable for this purpose. This procedure necessitated a condensation in the 2 layer system, chloroform-water (containing an alkali, preferably sodium carbonate). Good stirring or shaking is important during the reaction. The following combinations were studied: diazotised cholesteryl-4-aminobenzoate with histidine, carnosine, and histamine. The reaction products are soluble in chloroform, the color red to orange-red. Under analogous conditions tyrosine and tyramine gave a yellow condensation product. Experiments with egg white, edestine, and serum are in progress. Because of the relative ease with which diazotised cholesteryl amino-benzoate was found to condense with beta-naphthol this reaction was used in determining the best conditions for diazotisation. The resulting dye can be obtained in crystalline form when butyl alcohol is used as a solvent.

In analogy with experiments by Golodetz,[9] who prepared cholesteryl salicylate, the 3-nitro-salicylate was synthesized and reduced with a Pt-catalyst to the corresponding 3-amino-derivative. Difficulties were encountered in diazotising this substance, possibly due to the vicinal position of the substituents in the aryl radical.

This study is continued with the intention of extending it to other lipoidal substances.

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* Shriner and Ko prepared the hydrochloride by passing HCl through ether solution of cholesteryl-4-aminobenzoate and also reported insolubility in water.

[9] Golodetz, *Chem. Ztg.*, 1906, **31**, 1215.

6034

Preparation of Thyroglobulin.

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The method for preparing thyroglobulin has not been modified since Oswald described the procedure in 1899. He made saline extracts of fresh thyroid glands and precipitated the protein with an equal volume of ammonium sulfate. The process was time consuming since the precipitate had to be washed many times. Any other globulins present were also carried down and contaminated the final product. In attempting to prepare hog thyroglobulin, I found that the final preparation was frequently insoluble in saline or even dilute alkali. It was desirable to develop a more rapid method which would give a product suitable for intravenous injection. This has been accomplished by precipitating the thyroglobulin at the isoelectric point.

The fresh glands may be extracted with 0.1 M sodium acetate and the protein precipitated with dilute acetic acid. It has been found that sodium acetate will remove just as much of the iodine from fresh glands as sodium chloride. The optimum mixture for precipitation can be determined by setting up the following series of tubes:

Tube	1	2	3	4	5	6
cc. H_2O	8.75	8.5	8	7	5	1
cc. 0.1 N acetic acid	0.25	0.5	1	2	4	8

To each tube is added 1 cc. of the sodium-acetate extract. In a few minutes the optimum precipitate can be observed. It has been found that the maximum quantity of iodine is precipitated in the tube giving the greatest quantity of precipitate. For hog thyroglobulin, tube No. 3 gives the best results. Using these proportions, a considerable quantity of extract can be precipitated at one time.

If a soluble preparation is desired, it is necessary to remove the precipitate and redissolve it in a short time. If it is allowed to stand for 24 hours, the protein is denatured and cannot be redissolved. However, after one or 2 hours, considerable precipitate has settled out. Most of the supernatant fluid can be withdrawn and the remainder centrifuged. The precipitate is dissolved in 0.1 M sodium acetate by adding a little NaOH to maintain neutrality. The product

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can be further purified by reprecipitation 2 or 3 times. If one is not interested in preparing a soluble preparation, the yield can be greatly increased by allowing a longer time for precipitation with the acetic acid.

This procedure has several advantages over that of Oswald. The time is greatly reduced since no filtering or washing is necessary. The hemoglobin is not precipitated as it is with ammonium sulfate. The dilution 10 times and the slow formation of the precipitate allow many impurities to remain in solution. The cost is negligible even on a large scale. The isoelectric point is being used to purify thyroglobulin.

6035
Colony Forms of B. Paratyphosus B as Related to Variations in Gas Production.

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It is the purpose of this paper to describe variations in colony form and gas production that occurred in a stock culture of *B. paratyphosus B.* and particularly to emphasize a lack of correlation between these 2 properties.

Variations in properties of organisms of the paratyphoid group have been the subject of numerous studies. Jordan[1] has investigated colony form and its relation to virulence. Variation in the immunological properties of this group has been studied by the English workers.[2] Biochemical variations, particularly the failure of certain strains to form gas from carbohydrates was noted by Oette.[3] Herrmann[4] more recently isolated 16 strains which failed to form gas from dextrose and other fermentable carbohydrates.

During the course of experimental work irregular results in gas production from dextrose by a stock strain of *B. paratyphosus B.* led us to plate out the culture. The colonies were predominantly of the R type with a scattering of the S variety. A preliminary study of cultures of the S and R varieties with respect to their ability to form gas, resulted in subdividing both the R and S forms into

[1] Jordan, *J. Am. Med. Assn.*, 1926, **86**, 177.

[2] White, P. Bruce, A System of Bacteriology, London, 1929, V. 4.

[3] Oette, *Centralbl. f. Bakteriol.*, I. O., 1913, **68**, 1.

[4] Herrmann, *Centralbl. f. Bakteriol.*, I. O., 1929, **113**, 108.

strains which produced gas from dextrose and strains which failed to show this physiological activity.

As a result we had on hand 2 rough colony types Rg+ and Rg— which respectively did and did not form gas from dextrose. Also the cultures Sg+ and Sg— varied from each other in an analogous manner. These variants have been carried on solid medium for 4 months with no apparent change in investigated properties.

The biochemical properties: All forms produced acid from dextrose, maltose, mannite, galactose, xylose, and levulose and failed to produce acid from lactose, sucrose, raffinose, dextrin, and inulin. The fermentation tests were made both on carbohydrate Andrade agar and in carbohydrate broth. The variants Rg— and Sg— which failed to form gas from dextrose also failed to form gas from the other carbohydrates which they utilized with acid production. The variants Rg+ and Sg+ formed gas from all utilizable carbohydrates. No difference was noted in the rate of acid production of the variants in dextrose. All forms darkened lead acetate medium equally. Gelatin was not liquified after 16 days. No form produced indol. In indicator milk all forms produced an initial acidity without coagulation. The S variants caused a shift in the milk reaction to the alkaline range after 8 days incubation. An acid reaction was still present in the milk inoculated with the R form after 16 days.

All forms were agglutinated in a dilution of 1-1600 by a commercial anti-para B. serum. No significant agglutination was noted with anti typhosus or anti para-typhosus A. sera.

Morphologically the variants were characterized as Gram negative, non-spore-forming, motile rods.

To study the change in biochemical and colonial properties of the various forms, they were inoculated into mediums as indicated in the table and plates were streaked from these cultures at designated intervals. Colony forms were noted and 10 single colonies were subcultured to dextrose broth to determine the percent of gas-forming organisms present in the aging culture of the variant.

But limited changes in colony form of the cultures were noted during these studies. Intermediate forms, to the extent of 5-10% and an occasional S colony, occurred on plates inoculated from the aging R cultures. Intermediate and rough colony types appeared to a limited degree on plates streaked from the S. cultures. On further examination of the colonies for gas-forming cultures, as indicated above, less than 1% of the 500 colonies studied, arising from subcultures of the aging Sg+ and Rg+ forms failed to form

gas. These gas negative cultures arose from colonies essentially identical with those of the inoculated gas-forming type. The colonies occurring on these plates which were atypical of the inoculated form produced gas in all cases tested.

The non-gas-forming variants after 8 or 10 days incubation be-

TABLE I.

Percent of Colonies from Aging Cultures of Variants which Produced Gas when Inoculated into Dextrose Broth.

Date Inoculated	10-21-31			10-25-31			11-24-31			1-12-32		
Medium	100 cc. Plain Broth			10 cc. 20% Serum Broth			100 cc. Plain Broth			100 cc. Plain Broth		
Variant	Sg+	Sg—	Rg+ Rg—	Sg+	Sg—	Rg+ Rg—	Sg+	Sg—	Rg+ Rg—	Sg+	Sg—	Rg+ Rg—
Age of Culture day												
1	100	0	100 0	100	10?	90 0				100	0	100 0
2	100	0	100 0				90	0	100 0	100	0	100 0
4										100	0	100 0
6				90	0	100 0	100	0	100 0			
10	100	50	90 0	100	50	100 0				100	0	100 0
15										100	90	100 40
18	100	100	100 30	100	90	100 10	100	90	100 70	100	90	100 100
28	100	100	100 60	100	30	100 20	100	100	100 90			
52	100	100	100 100									

gan to yield an increasing number of colonies which, when examined, indicated the presence of gas-producing organisms. There was a lack of correlation between changes in colony form and gas production. Colonies atypical of the inoculated variant might or might not yield a gas-producing culture. The same was true for the colonies of the type inoculated.

Conclusion. The evidence presented seems to be in accord with the concept that bacterial properties may vary independently of each other.

6036

A Reducing Substance in the Urine of Cats Under Nembutal Anesthesia.

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A series of 15 cats were given intraperitoneal injections of nembutal (sodium iso-amytal, penta-barbital), the average dose being 60 mg. per kilo. Urine samples were collected at intervals after the injection and later analyzed for reducing substances by Sumner's method.[1] In certain instances the results thus obtained were checked by the use of Benedict's quantitative reagent. Simultaneously blood samples were taken and later analyzed for "blood sugar" by the Randle-Grigg modification of the Folin-Wu Micro Method.[2] These observations were made in the course of experiments to determine the effect of stimulation of the *tuber cinereum* upon carbohydrate metabolism. Rather extensive surgical procedures under deep nembutal anesthesia were required in the experiments. The bladder was exposed and emptied and the urethra cannulated from one to 2 hours after the injection of nembutal. The first sample of urine was taken about an hour after the urethra was cannulated and from 2 to 3 hours after the administration of nembutal. This sample always showed the presence of an abnormal quantity of reducing substance, usually the maximum obtained during the experiment, the concentration decreasing progressively in subsequent samples. This maximum reduction where the lowest maximum was obtained, was equivalent to that produced by 240 mg. % of glucose. In the experiment where the largest maximum was found, the reducing sub-

[1] Sumner, *J. Biol. Chem.*, 1925, **65**, 383.

[2] Randles, F. S., and Grigg, W. K., *J. Am. Med. Assn.*, 1924, **82**, 684.

stance was equivalent to that produced by 1575 mg. % of glucose. An average maximum value, equivalent to that produced by 1045 mg. % of glucose, was obtained.

The amount of reducing substance in the urine was definitely related to the time interval following the injection of nembutal and not to the "blood sugar" concentration. Often in the later stages of the experiment the reducing substance would be decreasing in amount while the blood sugar was increasing.

In Cat C the blood sugar remained at approximately a constant level of 150 mg. % throughout the experiment. Two hours after the initial injection of nembutal, the urinary concentration of reducing substance was found to be 961 mg. %. Forty-five minutes later a second injection of nembutal was given equivalent to 10 mg. per kilo body weight. Another sample of urine was taken 45 minutes later and the concentration of reducing substance had risen to 1428 mg. %. The third urinary sample was taken an hour later and the concentration of reducing substance had fallen to 735 mg. %, falling to 657 and 471 mg. % in the following 2 intervals of an hour and an hour and 20 minutes respectively.

These results suggest that the reducing substance may be due to the injected nembutal. Swanson and Shonle[3] state that the exact mode of elimination of this drug is not known, that examination of the urine failed to reveal any trace of the drug administered. We have found that nembutal when added to normal urine does not reduce the standard reagents used for the detection of reducing substance in the urine. It is, of course, unlikely that nembutal increases the permeability of the kidney to sugar, and subsequent fermentations of urine of nembutalized cats failed to show the presence of more than a negligible amount of sugar. The possibility, however, is not excluded that the reducing substance in the urine may be a product of the break-down of nembutal in the body.

[3] Swanson, E. E., and Shonle, H. A., *J. Lab. and Clin. Med.*, 1931, **16**.

6037

Comparative Value of "Gastric Mucin" and "Alkalies" in Prevention of "Peptic" Ulcer in Biliary Fistula Dogs.

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Fogelson[1] and Atkinson[2] have observed clinically that the administration of "gastric mucin" to gastro-duodenal ulcer patients was followed by a relief of the ulcer symptoms and an increase of body weight. Kim and Ivy[3] found that one ounce of "gastric mucin" a day prevented the occurrence of duodenal ulcer in biliary fistula dogs, in which the incidence of duodenal ulcer is from 40%[4] to 60%,[5] and that the animals on "gastric mucin" maintained their body weight much better than the control dogs not receiving mucin. It is well known that the administration of alkaline powders is of benefit in the management of patients with gastro-duodenal ulcer. We desired to ascertain if the administration of alkaline powders would have the same effect as "gastric mucin" in preventing the occurrence of duodenal ulcers and in maintaining body weight in biliary fistula dogs.

Ten healthy dogs were used in each series. The biliary fistula was made according to the method of Rous and McMaster.[6] All the animals were kept in single cages and were fed a stock diet of cooked yellow corn meal, bone soup and bread with mucin or alkali twice a day. The dose of "mucin" was 15 gm. with each meal. The dose of alkaline powder consisted of 1 gm. each of sodium bicarbonate and calcium carbonate with each meal. This dose of alkali was used because it has a slightly greater "buffering action" than 15 gm. of the "mucin" used in these experiments. All of the animals showed an absence of bile in the feces during life and in the stomach and duodenal contents immediately after being sacrificed. They secreted daily from 5 to 15 cc. of bile per kilo body weight, which is "normal" for biliary fistula dogs.[3] All were sacrificed at the time indicated in the table, the "alkali" dogs being sacrificed

* Eli Lilly Fellow.

1 Fogelson, *J. Am. Med. Assn.*, 1931, **96**, 637.

2 Atkinson, *J. Am. Med. Assn.*, in press.

3 Kim and Ivy, *J. Am. Med. Assn.*, 1931, **97**, 1511.

4 Kapsinow, *Ann. Surg.*, 1926, **83**, 614.

5 Berg and Jobling, *Arch. Surg.*, 1930, **20**, 997.

6 Rous and McMaster, *J. Exp. Med.*, 1923, **37**, 11.

TABLE I.

Comparison of the Effect of "Gastric Mucin" and "Alkalies" on Biliary Fistula Dogs.

No. of Dog	Length of Experiment—Days	Appetite and General Condition	Gastro-duodenal Ulcer	Loss of Weight %	Remarks
			With Mucin		
1	70	Very good	None	15	
2	49	Fair	"	25	
3	32	Good	"	22	
4	37	"	"	20	
5	116	Very good	"	15	
6	96	" "	"	15	
7	49	" "	"	15	
8	113	" "	"	12	
9	62	" "	"	15	
10	44	" "	"	18	
Average				17.4	
			With Alkalies		
1	31	Fair	None	33	
2	32	Good	"	33	
3	31	Very poor	"	56	Trophic ulcer on back Corneal ulcer. Bloody stool
4	31	Good	"	27	
5	32	Very poor	"	43	Autopsy shows gastroduodenitis
6	47	" "	"	56	Trophic ulcer on back Bloody stool
7	43	Poor	"	32	Trophic ulcer on legs
8	80	Very good	"	15	
9	63	Poor	"	42	Trophic ulcer on legs
10	50	Good	"	25	
Average				36.2	

earlier on the average because of the marked loss of weight. The period of observation was not too short in this experiment because the duodenal ulcer may develop in biliary fistula dogs in from 12 to 16 days after operation when fed only the stock diet.[3, 5]

A gastric analysis was performed 4 hours after the ingestion of the meal with mucin or the "alkalies". The average acidity in the "mucin dogs" was 8 units free and 80 total; in the "alkali dogs" the acidity was 0 units free and 35 total; on the "stock diet" without "mucin" or "alkali" the acidity was 27 units free and 75 total.

It was found that none of the dogs developed ulcers; but the dogs on "gastric mucin" did much better in regard to appetite, general condition and maintenance of body weight than the dogs on alkaline powders.

6038

Effect of Hypophysectomy on the Molar of the Rat.

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The gross and microscopic anatomy of the molars was studied in 2 groups of rats:

A. Completely hypophysectomized. 22 animals. Age at operation: 36 to 64 days. Post-operative life: 63 to 459 days. B. Litter mate controls. Some were unoperated but most were unsuccessfully subjected to operation. Normal oestrous cycles and normal rate of growth. 15 animals.

Significant alterations were observed only in group A. The gross findings in these animals were (a) retarded eruption; (b) shorter length of roots. The histologic findings are summarized below.

1. The epithelial attachment is absent in a number of cases for varying lengths near the cemento-enamel junction so that the enamel is bounded directly by connective tissue or by a cementum spur of varying length and width. In 7 animals the enamel shows here some areas of resorption.

2. The enamel is normal in structure except for areas of resorption near which the organic matrix is sometimes seen in decalcified sections.

3. The dentin appears, as a whole, normal in structure. It is resorbed when the resorption of the enamel or cementum has extended beyond the dentin boundary.

4. The pulp usually shows a reduced blood supply. In some cases it shows advanced atrophic changes and contains calcified globules. The size of the pulp chamber and pulp canal appears to be larger than normal.

5. The cementum shows an abnormal frequency of resorption which is most prominent at the bifurcation level and least prominent at the apical portion of the root. In some of the animals of longest post-operative life a great number of globules of various sizes that stain like the cementum are seen at the cementum surface which faces the periodontal membrane. In some cases similar but larger globules are seen in the periodontal membrane. These globules appear to be arranged in the direction of the periodontal membrane fibers. Cementum apposition is found to be continuous throughout the entire post-operative life.

6. The periodontal membrane contains an abnormal increase in the number of epithelial rests. These are usually proliferative and larger than normal. In advanced cases some of them are found to form into small cysts or to become calcified. The blood supply is below normal.

7. The alveolar bone shows an increased number and crowded arrangement of cementing lines. Bone apposition is found to be continuous throughout the entire post-operative life.

The actual conditions in a given case are progressive and thus depend on the time elapsing between the operation and the death of the animal.

6039

A New Method for Determining Intravenous Pressure.*

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Intravenous pressure may be estimated clinically either by direct or indirect methods. Direct methods, while more accurate, have certain disadvantages—impossibility of frequent determinations in the same patient, the necessary prerequisite of strict asepsis, the possibility of clot formation in the needle, the difficulty sometimes encountered in puncturing a vein in obese persons, and occasionally apprehension of patients which results in an elevated venous pressure.

The indirect or bloodless methods are free from most of these objections but the prevailing procedures have other disadvantages. The most serious objections are that the end-point is not sharp and correct estimations are therefore difficult, that the impossibility of applying such methods to obese persons in whom the veins are not visible above the surface of the skin, and that the end-point must be determined quickly.

The method we have developed is based on the principle that the superficial veins on the dorsum of the hand can be visualized easily in a darkened room by placing a small light, such as an ordinary pocket flashlight, against the palmar surface, preferably in the inter-

* Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

osseous spaces. By this transillumination the superficial veins appear as black bands which can be obliterated by moderate pressure, thus distinguishing them from shadows cast by ligaments or bone. The mouth of a small glass funnel is covered by thin rubber dam held securely in place by thread tied tightly in a previously prepared groove near the rim. A small hole is then cut in the center of the rubber dam through which the vein to be examined is visualized. Such a vein is previously selected in a darkened room in the manner indicated and its location marked. The surface of the rubber dam is then covered with rubber cement and the funnel is placed on the dorsum of the hand so that the previously selected vein is seen through the aperture in the rubber. It is held in place by moderate pressure until the rubber is adherent to the skin. This makes an air-tight seal. The tip of the funnel is then connected by rubber tubing to a suitable water manometer which is so graduated that pressure may be read in millimeters of water. The room is again darkened or a black cloth placed over the head of the observer, and the light is placed against the palmar surface of the hand in order to visualize the dorsal vein within the aperture of the rubber dam. The air pressure is then slowly raised in the instrument by a suitable rubber bulb—with the leak valve shut—until the vein under examination becomes obliterated as a result of the external compression. It is important to distinguish 2 stages—first, the obliteration of the vein, and second, further blanching of the skin. We have selected the former as the end-point at which the pressure is read off in the manometer in millimeters of water. Two observers were used—one to read the end-point, the other the manometer, and several readings were taken on each patient. A series of 12 patients were examined

TABLE I.

Name	Diagnosis	Direct Reading (H_2O)	Indirect Reading (H_2O)
H.S.	Rheumatic, mitral stenosis and insufficiency. Moderate decompensation	135	135
I. R.	Normal	55-60	50
B.P.	Non-cardiac	70	75-80
A.C.	Non-cardiac	65	65
K.	Hypertensive heart disease. Compensated	45	40-45
S.P.	Non-cardiac	65	60-65
C.M.	Non-cardiac	60	70-75
W.L.	Non-cardiac	90	105
J. L.	Non-cardiac	60	60
A.S.	Hypertensive heart disease. Moderate decompensation	110	115
G.D.	Non-cardiac	85	85-90
R.G.	Hypertensive heart disease. Obesity. Slight decompensation	100	90

by this procedure and the results compared with those obtained by the direct method.[1] These included patients with and without cardiac disease. An obese patient, whose veins were not visible above the surface of the skin, is included in this series. The table shows that a high degree of accuracy is possible when the results of our indirect method are compared with those obtained by the direct method.

We are presenting this preliminary report for the purpose of describing a new principle in the estimation of venous pressure. We believe that further experimentation will lead to improvements in the instrument and simplification of technic so that the method may be adopted for general clinical use. The advantages over other indirect methods are its greater accuracy, the simplicity of the apparatus, the absence of haste in reading the end-point, and the possibility of its use in obese and other patients where other indirect or direct procedures may fail.

6040

A Note of the Corneal Anesthesia Produced by Pilocarpine Administration.

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(Introduced by C. I. Reed.)

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The mydriasis which the administration of pilocarpine causes in the rat (Waddell,[1] Koppanyi[2]) is one of the numerous paradoxical responses elicited by this drug. During the course of an investigation on the nature of iris sphincter tone in this animal, it was noticed that the topical or subcutaneous administration of pilocarpine resulted in corneal anesthesia. Inasmuch as it has been shown that substances capable of acting as surface anesthetics would cause the pupil of the rat to dilate (Barnard[3]) it was thought, at first, that we held the key to the explanation of the pilocarpine mydriasis. Certain facts, however, indicate that pilocarpine does not owe its pupillo-dilator properties to the corneal anesthesia following its ad-

[1] Katz, L. N., Hamburger, W. W., and Rubinfeld, S. H. Observations on Oxygen Therapy. II. Measurements of Respiration and Circulation. In press.

[1] Waddell, J. A., *J. Pharm. Exp. Therap.*, 1926, **27**, 247.

[2] Koppanyi, T., and Sun, K. H., *Am. J. Physiol.*, 1926, **78**, 358.

[3] Barnard, R. D., *Am. J. Physiol.*, 1928, **74**, 407.

ministration. The mydriasis is of a greater extent than that following the application of some local anesthetic. Furthermore, pilocarpine constricts the pupil of the mouse, whereas local anesthetics such as hexylresorcinol and butyn dilate the pupil in this animal as they do in the rat (Heidgen and Barnard[4]).

Using graduated hairs for stimulation of the cornea, and accepting an absence of the winking reflex as a criterion for anesthesia, the effect of subcutaneous administration of pilocarpine was determined.

The number of experimental animals was, 34 albino rats, 9 albino mice, 5 guinea pigs, and 2 rabbits. Before the administration of pilocarpine the application of a pledget of cotton to the cornea would result in a closing of the eye, it being sufficient merely to touch the cornea very lightly. All animals were thus tested both before the administration of pilocarpine and after the removal of the pilocarpine anesthesia by atropinization. This constituted the control.

No definite number of stimuli were applied to the cornea in any instance, the usual procedure being to start the stimulus with the finest hair (one which would always produce winking in the non-pilocarpinized animal) and if this proved ineffective a coarser hair was used until an effective stimulus was obtained. As a rule, 5 hairs were used and this would therefore constitute 5 stimuli, applied however at a sufficiently great interval to render the possibility of summation remote.

In every instance, the administration of the drug resulted in an abolition of the corneal reflex within 5 to 60 minutes, depending on the dosage. That the failure to respond to the stimulation of the cornea was not due to paralysis of the extrinsic ocular muscles was evident from the fact that the animals would frequently wink spontaneously during the interval between impotent stimuli.

The pilocarpine anesthesia is removed by the subsequent administration of atropine (0.5 mg.-kilo) and is ineffective in causing corneal insensibility when given to an atropinized animal.

[4] Heidgen, M. F., and Barnard, R. D., *Am. J. Physiol.*, 1931, **92**, 276.

Missouri Section.

Washington University School of Medicine, February 10, 1932.

6041

Further Studies on the Heart and Median Cardiac Nerve of Limulus Polyphemus.

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The heart of Limulus consists essentially of 2 divisions, an atrium and a ventricle, each of which are contractile. The electromyograms obtained with the cathode ray oscillograph as a recording mechanism indicate that the fully contracted state is reached by a process of successive additions. In the atrial and ventricular muscle fibers the greatest amount of shortening occurs at the onset of a contraction, the further additions being of smaller and more nearly equal degree. The electromyogram consequently has a form somewhat comparable to the RST complex of the mammalian electrocardiogram. In contrast the electromyogram of a small group of isolated ventricular muscle fibers after stimulation with a single shock has the form of an ordinary diphasic muscle record, the rate of progression being 1 to 2 cm. per second.

The electroneurogram of the median cardiac nerve shows 2 groups of oscillatory discharges beginning 30 to 80 sigmas before the start of electrical activity in the musculature of the atrium and ventricle. The start of the ventricular electromyogram precedes the start of the visible ventricular contraction by several hundred sigmas. The oscillatory discharge can be shown to consist of potentials derived from 2 sources. From a correlation of the electrical and histological studies it is inferred that these 2 sources of potential are the axons of the large ganglion cells and the axons of the small ganglion cells. For reasons previously presented (Heinbecker) the large ganglion cells are considered normally to be the pace-maker cells, the small ganglion cells being responsible for the nerve impulses which directly innervate the cardiac muscle. Normally activ-

ity in the adult heart of Limulus is directly neurogenic in origin. The heart muscle can, however, be shown also to be rhythmically contractile without the intervention of cardiac ganglion cells. Under such circumstances conduction is peristaltic in type and its propogation rate is 1 to 2 cm. per second. In the normally innervated heart, contraction is practically simultaneous throughout its length. The chronaxie of the denervated ventricle in air when recorded by point electrodes is of similar order to that of the chronaxie of the normally innervated ventricle.

The heart rate ordinarily may be decreased or increased by the action of the extrinsic nerves. This is indicated by the effect of destruction of their central connections. Stimulation of the extrinsic nerve centers after removal of the median cardiac nerve is without effect on the heart rate or the irritability of the ventricular musculature. It therefore is inferred that the extrinsic nerves act only on the ganglion cells of the heart and not on the heart musculature directly.

Stimulation of the extrinsic nerve fibers of the heart alters the potentials derived from the median cardiac nerve. The effect of the 'vagus' fibers is to reduce the amplitude of the potential, to show its oscillatory rate and shorten its total duration even to the point of extinction. The effect of the 'sympathetic' fibers is to increase the amplitude of potential (greater synchronization of ganglion cell responses), to increase the frequency of the oscillatory discharges and to lengthen their total duration. The extrinsic nerves modify the rate of occurrence of the discharge complexes. They also modify the time interval between the complexes which initiate contraction of the atrium and the ventricle.

From the above and other experimental evidence not here reported, it appears that changes in the heart rate may be effected by the activity of fibers which modify the intrinsic rhythm of the large ganglion cells. The axons of these cells in turn modify the rate of discharge of the small ganglion cells whose axons stimulate the heart muscle directly. This alteration in rhythm of the small ganglion cells by the large ones is only in a positive direction from their own autochthonous rhythm. Since by the activity of the 'vagus' extrinsic nerve fibers it is possible to depress all potentials of the median cardiac nerve to extinction, it follows that the extrinsic nerve fibers must also act directly on the small ganglion cells as well as on the large ones. Our experimental evidence also indicates that the 'sympathetic' fibers influence directly both small and large ganglion cells.

6042

Vitamins B_1 and B_2 in Tissues of Normal and Experimental Rats.

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The relative concentration of Vitamins B_1 and B_2 in normal and experimental rats was determined by feeding dried rat tissue as the source of these vitamins. "Normal rat tissue" was prepared from stock rats, 200-300 gm. in weight. The rats were decapitated, the carcasses (except skin, feet, tail, and intestines) were hashed in a meat grinder and the hashed material was dried in a warm air dryer (50°C.). In the same manner "B_1 rat tissue" was prepared from 20 rats after 30 days on the Evans and Burr diet supplemented with cod liver oil and tikitiki. The average starting and final weights of this group were 186 gm. and 172 gm. respectively, indicating cessation of growth and a loss of weight during the 30-day period on the diet deficient in B_2. "B_2 rat tissue" was similarly prepared from 20 rats after 30 days on the Evans and Burr diet supplemented with cod liver oil and autoclaved liver. The average starting and final weights of this group were 184 gm. and 148 gm. respectively, indicating cessation of growth and a loss of weight during the 30-day period on the diet deficient in B_1. These 3 rat tissue preparations were fed as sources of B_1 and B_2 to young 50 gm. male rats fed the Evans and Burr diet supplemented with cod liver oil and either tikitiki or autoclaved liver.

Normal rat tissue was not a good source of either B_1 or B_2 but was definitely richer in the latter. Better growth resulted with 0.3 gm. of this tissue as a source of B_2 (Group 8) than with 0.7 gm. as a source of B_1 (Group 7).

Normal rat tissue contained more B_1 than either the B_1 or the B_2 rat tissue preparations. The rats fed the B_2 rat tissue as a source of B_1 (Group 11) failed to survive unless tikitiki was added, the results resembling those obtained with autoclaved liver alone (Group 1). The feeding of B_1 rat tissue as a source of B_1 (Group 9) permitted survival but there was no growth after the first 10 days. It was evident from these experiments that the bodies of the rats used in the preparation of the B_1 and B_2 rat tissues were practically depleted of vitamin B_1 during the preliminary 30-day period.

These results were in marked contrast to those obtained when the same tissue preparations were used as a source of vitamin B_2.

Normal (Group 8) and B_2 (Group 12) rat tissue were of nearly equal value as sources of B_2. The concentration of this factor in B_1 rat tissue (Group 10) was lower than in the normal rat tissue but enough was present to permit growth. The rats used in the preparation of the B_1 rat tissue showed partial loss of appetite, failure of growth and loss of weight during the 30 day period on the diet containing B_1 and lacking B_2, yet the bodies of these rats still contained appreciable amounts of B_2 at the end of the 30-day period.

It was concluded that the tissues of rats on deficient diets may be readily depleted of B_1 but not of B_2. Whether this residual B_2 represents stored B_2, or B_2 which is conserved by the body and used sparingly is being investigated. The results are in accord with the observation of Osborne and Mendel[1] that livers of rats fed a diet deficient in the vitamin B complex could not supplement a diet deficient in the B complex and indicate, in addition, that the limiting factor in their experiments was vitamin B_1.

The interpretation of experimental results obtained with diets deficient in B_2 should include recognition of this residual B_2. The presence of this factor in the tissues may be partially responsible for the better appetite and for the resulting longer survival of rats fed tikitiki (Groups 2, 3, and 4) as the sole source of the vitamin B complex. Our results confirm the observation of Sherman and Sandels[2] that the appetite and food consumption of rats decrease more rapidly on diets low in B_2 than on diets low in B_1.

Our previous experiments[3, 4] have suggested that B_1 and B_2 are both appetite stimulants but only in the presence of each other. The present data introduce the possibility that the sustained although subnormal appetite of rats on B_2 deficient diets may be due to the presence of residual B_2 in the tissues whereas the sharp failure of the appetite of rats on B_1 deficient diets may be due to the fact that the tissues are rapidly depleted of B_1. In the former both factors would be present, in the latter only B_2. If dogs also retain tissue B_2 then the above possibility might apply to the experiments of Burack and Cowgill.[5] On the assumption that both factors, B_1 and B_2, are required for normal appetite, a stimulation of appetite of ani-

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[3] Graham, C. E., and Griffith, W. H., *J. Biol. Chem.*, 1931, **92**, lxiii.

[4] Graham, C. E., and Griffith, W. H., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1086.

[5] Burack, E., and Cowgill, G. R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 750.

TABLE I.

Group	No. of Rats	Gain in Weight in 30 days		Gain in Weight in 50 days		Average Total Calories	Tiki-tiki	Auto. liver	Special Supplements
		low-high	mean	low-high	mean				
		gm.	gm.	gm.	gm.	cal.	cc.	gm.	
1	20	(−4)-22	7*	—	—†	—	—	1.0	None
2	12	(−3)-25	11	(−6)-16	9	846	0.25	—	None
3	4	5-32	17	9-35	17	842	0.50	—	None
4	5	12-25	16	11-28	18	895	0.50‡	—	None
5	6	78-142	110	105-212	152	2088	0.25	0.5	None
6	6	90-105	99	122-158	148	2025	0.50	1.0	None
7	8	22-55	43	40-81	55	1107	—	0.5	0.7 gm. normal rat tissue
8	8	35-78	52	46-99	72	1290	0.25	—	0.3 gm. normal rat tissue
9	10	14-33	24	14-32	22	881	—	0.5	0.7 gm. B_1 rat tissue
10	9	25-56	40	22-64	50	1077	0.25	—	0.3 gm. B_1 rat tissue
11	10	(−9)-17	4	—	—	—	—	0.5	0.7 gm. B_2 rat tissue
12	10	32-65	48	51-85	66	1272	0.25	—	0.3 gm. B_2 rat tissue

* 16 survived. † 5 survived. ‡ Changed to 0.25 cc. on 30th day.

mals on a diet lacking the B complex might be expected from the administration of B_1 as long as residual B_2 was present in the tissues whereas negative results would follow the administration of B_2 due to the absence of tissue B_1. Obviously, in such a case it would be necessary to perform similar experiments on animals wholly depleted of residual or tissue B_2. The real significance of the residual B_2 must await further investigation.

6043

Electrical Activity of the Cerebral Cortex as Compared to the Action Potential of Excised Nerve.

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As Bartley and Newman have shown, there can be recorded from the cerebral cortex, over considerable of its surface, a continuous and extremely complicated series of electrical oscillations. The record as it appears on the cathode ray oscillograph, with adequate sensitivity to show its complexity, is far too intricate for any such casual analysis into specific waves as is possible in nerve trunks.

That this activity consists of a complication of more simple elements is indicated by the following experiments: If in the rabbit's or dog's cortex shallow cuts (1 mm. deep) are made near a recording electrode (an indifferent electrode being located on the bone of the skull), the form of the record changes, becoming simpler. Cutting off the blood supply from an area causes its activity to cease in 5 to 10 minutes, without any obvious simplification. Ether anesthesia may cause marked simplification of the record at a stage shortly before all activity ceases. The simplest large waves have a duration of 30 to 100 sigma, but in the complex picture there appear to be some as short as 10 sigma. The amplitude of individual waves of the normal record may be as much as ½ mv., increasing upon stimulation of the animal to several times this. No oscillations can be found even at a sensitivity of ½ meter per mv., that have as short a duration as nerve-fiber action potentials. The long refractory period, low frequency, long duration and suppression by anesthesia at a concentration that does not affect nerve fibers indicate that nerve cells spontaneously active in the cortex are involved, and suggest that the potentials themselves may be cell potentials rather than nerve-fiber potentials. Further, responses to direct electrical stimuli are too long in duration for nerve-fiber potentials, and are of the order of duration of the spontaneous waves.

It therefore seemed of interest to determine what the action potential of a simple bundle of nerve fibers would look like when recorded from the surface of the cortex or from beneath the surface. A rabbit vagus nerve ½ mm. in diameter was laid across the cortex, stimulated at one end and explored with a lead electrode. When

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the test electrode just touched the surface of the vagus the amplitude was 6% of that recorded when the vagus was lifted off the cortex. Laid in a slit of the cortex the amplitude was slightly less; it was 3% when embedded 1 mm. deep in the cortex, and not over 1% when threaded through the ventricle of the brain. It follows that if all the fibers in the cortex within 1 mm. of the test electrode were active at once, their summed potentials would be shunted by the rest of the brain tissue to not over 2 or 3% of what their value would be if insulated, and the record of fibers further away than this would soon become vanishingly small. Furthermore, to produce waves 30 to 100 sigma long these fibers would have to respond one after another or else repetitively, and in so complicated a record as the cortex gives, obviously not all are responding in any one wave.

Computing on this basis, it appears that to give a record as great as the largest of the waves recorded from the cortex after stimulation, for instance of the optic nerve, would require fibers whose activity equaled that of all the myelinated fibers of nearly one thousand vagus nerves, to be collected within recording range of the test electrode, for example, 2 mm. If each responded 3 times at, for example, 20-sigma intervals this number could be correspondingly less. Or, if each fiber in the cortex gave one potential equal to a 15-mu myelinated axon from the bullfrog's skin nerve, 250,000 such potentials would be required to form 1 cortical wave. The cross section of this bundle would be 44 sq. mm. if packed close, or over 7 mm. in diameter. Since the rabbit cortex is only 3 mm. thick, and contains many neuroglia and nerve cells as well as fibers, this would demand that some of these fibers entering into the record be over 5 mm. distant from the electrode, and at this distance they would not affect the record appreciably.

It thus appears either that the axons of the brain have a much larger potential than elsewhere, or else the record is due to nerve *cells,* having a higher and more protracted potential than nerve fibers give. On the other hand, it would be impossible at any sensitivity that is available at present (1,000 meters per volt) to detect less than five 15-mu fibers responding in a volley, even if immediately under the test electrode, and more would be required if their potentials were out of phase or their position further from the lead. This would have to be observed superposed upon an already very complicated record, whose waves at this sensitivity would, some of them, be one meter high. It is, therefore, presumable that the finer details of cortical activity are still escaping our notice.

6044

A Note on the Effect of Theelin, Theelol and the Luteinizing Substance on Reproduction.

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Past investigations have definitely shown that the prolonged administration or single massive doses of the female sex hormone exerts an inhibiting effect on the ovary. By means of ovarian transplants[1] and extracts[2] Haberlandt produced a lasting sterility in rabbits and guinea pigs. These results have been confirmed by Bondi and Neurath[3] and Reiprich,[4] who used ovarian transplants, and by Fellner[5] and Gostimirovic[6] with feminin and menformon-folliculin respectively. The effect on the ovary produced by high dosages of folliculin[7, 8, 9] has been duplicated with theelin by Doisy, Curtis and Collier,[10] who found in the immature rat a complete inhibition of growth of the ovary, degeneration of follicles and inhibition of the development of new follicles. Similar effects have been obtained by Hisaw, Leonard and Myers[11] upon injection of amniotin and by Kunde and coworkers[12] with the female sex hormone from pregnancy urine when administered to immature dogs.

The luteinization of the ovary by anterior lobe substance has been adequately demonstrated. Evans[13] found that the cessation of estrus in the normal rat resulting from the injection of large amounts of saline extracts of beef anterior pituitary glands was associated with a profound luteinization of the ovary and an embedding of the ova in the corpora lutea. This effect has been induced by other in-

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[2] Haberlandt, L., *Pflug. Arch. f. des. ges. Physiol.*, 1923, **202**, 1.

[3] Bondi, Josef, and Neurath, Rudolf, *Wien. Klin. Wochschr.*, 1922, **35**, 520.

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[6] Gostimirovic, D., *Biol. Z.*, 1929, **49**, 24.

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[9] Hauptstein, P., *Endokrinol.*, 1931, **8**, 169.

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[11] Hisaw, F. L., Leonard, S. L., Meyer, R. K., *Endocrinol.*, 1931, **15**, 17.

[12] Kunde, M. M., D'Amour, F. E., Gustavson, R. G., and Carlson, A. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 122.

[13] Evans, H., Harvey Lectures, 19, 1924.

vestigators.[14, 15, 16, 17, 18, 19, 20] Similar results have been obtained with extracts of pregnancy urine. Thus the injection of Prolan B into adult mice is reported by Zondek[20] to have caused cessation of the estrus cycle and luteinization of the ovaries. Since Prolan B causes all follicles to become luteinized, Zondek believes that ovulation is inhibited and hence a veritable sterilization is produced. In line with this, Engle[21] found that the treatment of immature mice with pregnancy urine resulted in atresia of the follicles and prevention of ovulation by the transformation of the follicle into a corpus luteum with retained ovum. That a sterility is actually induced has been shown very recently by Mandelstamm.[22]

Experimental. Although the effect of these substances upon the ovary seems to be quite amply established, it seemed desirable to determine the permanency of the inhibition produced by daily administration of the crystalline hormones theelin and theelol and also of a purified preparation of the luteinizing substance of pregnancy urine. Four groups of 5 healthy, 120-day-old, female rats with normal cycles were injected for 25 days. The first group was injected daily with 50 mouse units of the luteinizing substance, the second group with 6 spayed rat units[23] of theelol, the third with 6 immature rat units[23] of theelol, and the fourth group with 6 rat units of theelin. Smears were made daily throughout the course of the experiment. The animals remained in good health during the entire period of treatment.

The rats which received the luteinizing extracts were in a continuous state of estrus for the first 5 to 7 days of the treatment, whereupon estrus was in all cases completely inhibited. After this inhibition, which persisted for 5 to 10 days following the cessation of the injections, the reestablished cycles were of greater than normal length due to a long diestrus interval. The animals receiving theelin and theelol stayed in continuous estrus throughout or evidenced very long cycles with extremely short dioestrus intervals.

[14] Parkes, A. S., *Proc. Roy. Soc.*, 1929, **B104**, 171.
[15] Zondek, B., and Aschheim, S., *Arch. f. Gyn.*, 1927, **130**, 1.
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[17] Fels, E., *Wien. Klin. Wochen.*, 1928, **41**.
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[20] Zondek, B., *Die Hormone des ovariums und des Hypophysenvorderlappens*, 174.
[21] Engle, E. T., *J. Am. Med. Assn.*, 1929, **93**, 276.
[22] Mandelstamm, A., *Zentralbl. f. Gyn.*, 1931, **55**, 3004.
[23] Curtis, Jack M., Doisy, E. A., *J. Biol. Chem.*, 1931, **91**, 647.

The cycles returned to their normal rhythm promptly after the injections were stopped.

At the end of the 25-day injection period, 2 animals from each group were sacrificed for observations of the genital tract. (Table I.) The ovaries of the group injected with the luteinizing principle

TABLE I.

Rat No.	Total Amt. Injected	Period of Injection, Days	Weight of both ovaries, mg.	Follicles	Corpora Lutea	Degree—enlargement of uterus
			Luteinizing Hormone (Mouse Units)			
38	1250	25	372	10 (large to medium)	100-150	enormous (gravid type)
40	1250	25	263	"	"	"
			Theelol (Spayed rat unit = 0.68γ)			
43	150	25	41.1	12 (small)	15	moderate
44	150	25	67.8	10 "	20	"
			Theelol (Immature rat unit = 0.16γ)			
47	150	25	74.4	5 (small)	20	"
51	150	25	43.7	3 "	15	"
			Theelin (Rat unit = 0.33γ)			
52	150	25	60.3	a few (small)	25	"
53	150	25	50.4	"	20	"

were enormously enlarged (5 and 7 times normal), the bulk of the weight obviously was made of lutein tissue although several follicles, varying in size from large to medium, were present in each case. The uteri were livid and very much enlarged, giving the appearance of pregnancy. This excessive luteinization apparently persists for some time for, upon examining the ovaries of rats 37 and 41 twenty-five days after the injections had been stopped and after the appearance of 3 and 2 cycles respectively, it was found that the luteinization was still pronounced although there was evidence of lutein degeneration. In rat 37 no follicles were in evidence while in rat 41 a few very small follicles were observed. When the ovaries of rat 41 were again inspected 40 days after the cessation of injections their appearance was very much the same as on the first examination except for a more marked degeneration of the corpora lutea.

There was little difference in the appearance of the genital tracts of the rats receiving theelol and those receiving theelin. The weight of the ovaries was very nearly normal even though a considerable number of very small and highly congested corpora lutea were present in all cases. The ovaries of the rats receiving the higher dosage of theelol had a greater number of follicles but in all cases their size

was exceedingly small. The uteri were only moderately enlarged.

A recovery period of 29 days was allowed before the males were admitted to the females. Copulation was determined by observing sperm in the vagina and the beginning of pregnancy was taken as the date when such a finding was made. This was confirmed by the absence of further estrus and also by the placental sign on the thirteenth day of pregnancy. The number of males and females in each litter was observed; also the age at which canalization of the vagina took place. The results are recorded in Table II.

TABLE II.

Rat No.	Total Amount Injected	Period of Treatment. Days	Recuperation Period Allowed. Days	Elapsed Time before Copulation Took Place. Days	Length of Gestation Period. Days	Number in Litter	Number Survived	Number of ♀	Number of ♂	Age at Which Vagina Opened Days
				Luteinizing Hormone (Mouse Units)						
37	1250	25	29	23	22	2	0	—	—	
39	1250	25	29	28	21	6	6	5	1	46-54-54-55-55
41	1250	25	45	4*	21	8	8	2	6	39-42
				Theelol (Spayed rat unit = 0.68γ†)						
42	150	25	29	17	22	10	7	3	4	45-47-49
45	150	25	29	4	22	9	9	3	6	37-39-41
46	150	25	29	9	22	7	7	3	4	42-42-50
				Theelol (Immature rat unit = 0.16γ)						
48	150	25	29	4	22	9	9	1	8	39
49	150	25	29	1	22	8	8	4	4	37-38-38-37
50	150	25	29	1	21	8	8	5	3	37-38-38-40-41
				Thœlin (Rat unit = 0.33γ)						
54	150	25	29	12	22	10	9	1	8	38
55	150	25	29	9	22	11	10	7	3	37-38-38-40-42-45-47
56	150	25	29	9	22	9	9	7	2	43-43-44-44-45-45-45

* Mated 16 days later than No. 37 and No. 39.
† γ —0.001 mg.

It will be observed that, with the exception of the rats receiving the smaller dosage of theelol, 29 days were not sufficient for complete recovery from the effect of the injections for they did not receive the male even though estrus occurred normally as indicated by the smear technique. The animals receiving the luteinizing substance recovered more slowly than did those injected with theelin and theelol. The length of the gestation period in each case was normal.

The young were normal and healthy and the survival was very good. The limited number of animals used in this experiment does not permit any conclusion regarding the ratio of males to females

in the litters. Canalization of the vagina took place at about the same age as it does with the young stock females of our colony.

Discussion. While a greater number of animals treated with both larger and smaller quantities of these hormones for longer and shorter periods of time is desirable, we believe that our data justify certain conclusions. It seems quite definite that the changes evoked by the administration of theelin, theelol and the luteinizing substance do not permanently affect the normal ovarian function. The fact that those animals receiving the smaller amount of theelol mated immediately upon introduction of the males might indicate that a sterilization was induced in the other 3 groups. However, since we have not determined the average length of time that untreated females remain with the male before fertilization takes place, such a conclusion may be unjustified.

Summary. Prolonged administration of either theelin or theelol to adult female rats does not destroy the reproductive function, as is evidenced by their ability to bear and rear normal litters. The treatment with the luteinizing substance from pregnancy urine produces complete inhibition of estrus together with profound ovarian luteinization but does not permanently impair the reproductive mechanism.

6045

On the Classification of Cells According to Their Inorganic Structure in vitro.*

E. S. HORNING† AND GORDON H. SCOTT. (Introduced by E. V. Cowdry.)

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One of the greatest difficulties in carrying out any problem *in vitro* is that of establishing a morphological criterion by which the various cell elements composing the growth can readily be classified. When dealing with pure culture strains of "Fibroblasts" and epithelial cells it is a comparatively simple matter to distinguish between the 2 cell types, as they not only exhibit distinct differences in their morphology, but also in their mode of growth. But when

* Aided by appropriation from a grant made by Rockefeller Foundation to Washington University for research in science.

† Rockefeller Foundation Fellow.

attempting to distinguish these 2 different types of cells in a mixed growth colony it becomes more difficult, as the epithelial elements composing the advancing free edge of the new growth invariably isolate themselves from the epithelial sheet and migrate into the culture medium as separate units, assuming a contour similar to fibroblasts (see Fig. 1). But far greater difficulties confront the

FIG. 1.

Representing inorganic structure of cells in the peripheral region of a 7 days' chick epithelial tissue culture, as seen by dark field illumination. Observe the diffuse distribution of calcium salts, and also the manner in which the cells on the free growing edge of the culture have become mobilized and have assumed a fibroblast-like contour.

investigator when he has to identify cells of the mesenchyme type, such as osteoblasts, chondrioblasts, and heart fibroblasts which, although possessing varying functional activities, exhibit similar morphological values.

Parker and Fischer,[1] realizing this difficulty, successfully demonstrated that mesenchyme elements in tissue cultures, which would be designated morphologically as "Fibroblasts", are found to possess different inherent growth potencies when cultivated under similar

[1] Parker, R. C., and Fischer, A., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 580.

conditions *in vitro*. On these grounds they rightly contended that "the physiological properties of the cell should constitute the first claims to any definition."

Recently Horning[2] found that tissue culture cells which exhibit the same morphological characteristics and behavior under normal conditions *in vitro,* differ, however, in their reactions to similar pathological conditions, as the rate of cytolysis was in all cases found to be dependent upon the inherent growth energy of the given strain.

To devise a more practicable method by which cells exhibiting different functional values, and expressing a similar morphology could easily be recognized, tissue cultures of such growths, that were isolated from the same embryo and incubated under similar conditions, were subjected to microincineration. The inorganic structure of the incinerated explants was studied in dark field illumination obtained by using a Zeiss cardioid condenser. The cultures were incinerated in an electric quartz oven at 650°C. in the manner described by Scott and Horning,[3] except that when employing tissue cultures it was found advantageous, for optical reasons, to explant the selected tissues upon a small glass slide, and mount the coverglass over the inorganic remains.

An examination of the inorganic properties of the cells forming the zone of new growth in a pure strain, shows no visible gradation in the mineral content between those elements nearer the original implant and those which have migrated the farthest into the liquid medium forming the growing edge of the culture. Similar observations, however, upon cultures of mixed growth, that were obtained from the digit of a 7 days' chick embryo, revealed a striking variation of the inorganic structure of the different types of cells. Clasmatocytes and other amaeboid elements are the most outstanding owing to the enormous concentration of mineral salts, both within the main body of the cell and in their pseudopodia. These elements are rendered most conspicuous by the large amounts of calcium oxide they possess, and stand out in this respect when compared with the other "Fibroblast" structures. The inorganic remains of those cells possessing the fibroblast-like contour reveal a visible difference in the changes and distribution of their mineral ash deposit. The mature and immature blood structures are easily detected, but their nuclei contain no appreciable traces of iron oxide. This also applies to the nuclei of all microincinerated tissue

[2] Horning, E. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, in press.

[3] Scott, Gordon H., and Horning, E. S., *J. Morph. and Physiol.*, in press.

culture cells. When compared with incinerated sections of chick embryos of the same age, whose nuclei are rendered most conspicuous by the concentration of iron oxide especially within the blood elements, the contrast is most apparent. This interesting phenomenon was also a prominent feature in cultures of pure tissue strains. This change of the inorganic salts in tissue cultures, after periods of growth *in vitro* is significant and might possibly be regarded as an interesting intrinsic chemotactic reaction to implantation in a foreign medium.

The ash deposit as seen by dark field illumination in pure cultures of epithelium and heart fibroblasts obtained from 7 days' embryonic chicks shows an interesting but slight variation of the mineral salts. The inorganic structures of the epithelial cultures are represented by a diffuse distribution of calcium oxide and apparently also of sodium. As a whole, the ash within these cells is less concentrated than that observed in sections of whole embryos of the same age. This might be due to the fact that cells *in vitro* lose their compactness as they are enabled to migrate freely and spread out under the surface of the coverglass. Cells on the extreme periphery of the epithelial sheet, that become mobilized, and assume a "Fibroblast-like" contour, show no difference or orientation in their mineral constituents (see Fig. 1). This is extremely interesting as it has been recently demonstrated, experimentally *in vitro,* that this apparent difference is not a true differentiation, but a superficial mechanism due to an effort on the part of the cell to increase its surface area owing to lack of oxygen.[4]

The heart fibroblasts contain considerably greater concentrations of calcium deposit than the epithelial cells and visibly less sodium. In mixed cultures it is possible after careful examinations to discriminate between the 2 cell types according to their inorganic structure. When these cells are compared with the incinerated remains of osteoblast cultures which were obtained from the supraorbital of an 8½ days' chick embryo, it is seen that these osteoblast structures contain such enormous concentrations of calcium salts, together with no visible trace of sodium, that their inorganic individuality becomes apparent.

This investigation is significant inasmuch as it has indicated that cells designated as mesenchymal elements expressing similar morphological values can be classified according to their inorganic properties.

[4] Horning, E. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, in press.

6046

Study of Normal and Malignant Tissues by Microincineration.*

GORDON H. SCOTT AND E. S. HORNING.† (Introduced by E. V. Cowdry.)

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Policard and Doubrow,[1] using the technique of microincineration, made a comparative study of corresponding normal and malignant tissues and found significant differences in their ashed remains. Recently it has been possible to improve the technique so that one can examine incinerated preparations with oil immersion objectives and study individual cells with great facility (Scott[2, 3]). Using these improvements, Horning and Scott[4] studied the distribution of the inorganic salts in the developing chick embryo and found some points of interest in connection with the Cohnheim "Embryonal Theory" of tumor proliferation. Our belief that the method of microincineration is capable of yielding information of a new kind relative to malignancy has led us to study some human medullary duct carcinomata of the breasts as well as several of the scirrhous types,‡ together with the transplantable mice tumors, M. 63, S. 37 and 180.

The preliminary observations of the microincinerated tumors confirmed the previous statement of Policard and Doubrow that cancerous tissue remains carbonized longer than does normal tissue. Another constant feature of the human and rodent neoplasms is the relatively greater content of ash of the tumor when it is compared with similar normal tissue. Observations with oil immersion objectives show that the nuclei contain more ash than do nuclei of normal duct tissue. This deposit is marginated and evidently represents the ash remains of an hyperchromatic condition similar to that described by Horning and Richardson[5] in malignant growths. It is interest-

* Aided by appropriation from a grant made by Rockefeller Foundation to Washington University for research in science.

† Rockefeller Foundation Fellow.

1 Policard, A., and Doubrow, S., *Ann. d'Anat. Path. med.-ch.*, 1924, **1**, 163.

2 Scott, G. H., *Comp. Rend. Acad. Sci.*, 1930, **190**, 1073.

3 Scott, G. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 349.

4 Horning, E. S., and Scott, G. H., *Anat. Rec.*, 1932, in press.

‡ We desire to express our thanks to Dr. Robert Elman, who supplied us with this material. Thanks are also due to Dr. Leo Loeb and Dr. F. Carter Wood for providing these rodent transplanted tumors.

5 Horning, E. S., and Richardson, K. C., *Med. J. Australia*, 1930, Feb. 22, 3.

ing to note that these nuclear salts contain visibly more iron oxide than do those of normal cells. The cytoplasmic ash deposit is more abundant than in the normal.

Three factors are suggested which cause the increased appearance of ash in the cancerous ingrowths when viewed with the low power of the microscope. There are more nuclei per unit area present than in the adjacent fibrous stromal tissue and the nuclei themselves contain more inorganic residue than do those of normal cells. In addition, the cytoplasm of the neoplastic cells contains more mineral salts than is common for this type of tissue.

A survey of the inorganic structure of neoplastic and normal tissues demonstrates that the individual cells composing the malignant growths are richer in mineral constituents than normal tissues —especially in calcium and iron oxide. An additional interesting feature is the similarity between developing embryonic cells and cancer cells with respect to the distribution and arrangement of certain mineral salts. Both types are characterized by an extraordinary variation in intensity, concentration and orientation of their inorganic constituents and contrast greatly with the appearance of the mineral elements in healthy adult tissue which remain proportionally fixed.

6047

Experimental Fixation of the Mediastinum.

MAURICE BERCK. (Introduced by Evarts A. Graham.)

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One of the difficulties in carrying out surgical compressive therapy for various lung diseases is that in some individuals the mediastinal contents are so little stabilized that such procedures as artificial pneumothorax and theracoplasty merely result in crowding the affected lung over into the opposite side of the chest instead of producing the desired compression of pulmonary cavities. The theoretical considerations, together with the demonstration of the importance of having a fixed mediastinum in such conditions, upon which is based the modern treatment of acute empyema have been described by Graham.

The method producing fixation must be innocuous, *i. e.*, should not produce "Pick's syndrome" of adhesive mediastino-pericarditis,

and furthermore the duration of the various types of fixation should be of differing degree.

The anterior mediastinal space of rabbits is potentially large with ready extension to the anterior portion of the superior mediastinum. This space accommodates 15 cc. of fluid. Roentgenograms of injections of this space with radiopaque fluids demonstrate the feasibility of filling this space with fluid substances which either by action as mechanical barriers or by evocation of a benign, slowly progressive, productive inflammation would secure fixation of the mediastinum in the mid-line sufficient to resist large alterations of intrapleural pressure.

The standard of control was the shift of the mediastinum (adult rabbits) in response to 20 cc. of air introduced into the left pleural cavity. Uniformly in the 6 control animals such a pneumothorax succeeded in shifting the mediastinum far over to the right so that the left cardiac border coincided on the roentgenograms with the left margin of the vertebral column.

The following injections of the anterior space and of the anterior portion of the superior space were made through a needle introduced on both sides of the xiphoid process: (1) 15 cc. 30% acacia solution (4 rabbits), (2) 15 cc. agar (6 rabbits), (3) 15 cc. mineral oil (6 rabbits), (4) 15 cc. suspension of silicon dioxide in mineral oil (3 rabbits), (5) 15 cc. suspension of silicon dioxide in water (2 rabbits), (6) 15 cc. suspension of sodium and potassium silicate in mineral oil (3 rabbits), and (7) 15 cc. suspension of sodium and potassium silicate in water (3 rabbits).

Autopsies performed on a number of the animals have shown the fixing material to be in the distended anterior space and anterior portion of the superior space. The acacia (30%) disappears entirely after 36 hours; the agar has remained for at least 1½ months; the mineral oil persists for several months and engenders a low grade fibrotic reaction; the silicon dioxide and silicate in oil evoke a more pronounced fibrotic reaction; the suspension of silicon dioxide and silicate in water arouses a more intense productive exudative mediastinitis.

The fixed mediastina were observed roentgenologically to be widened moderately and situated in the mid-line. After variable periods of time (6 hours to 4 months, dependent on type of fixatant used) left pneumothoraces were induced in the identical manner as in the control rabbits. Roentgenograms were then immediately taken.

The results as illustrated by the roentgenograms may be sum-

marized briefly as follows: 1. Fixation of the mediastinum in the mid-line sufficient to resist the pressure of a 20 cc. left pneumothorax, which in unfixated rabbits causes shifting of the mediastinum far to the right, can be secured by injection of the anterior mediastinal space and anterior portion of the superior mediastinal space with 15 cc. of 30% acacia or agar or mineral oil or suspension of silicon dioxide and silicate in mineral oil. 2. Complete fixation of differing durations can be secured. The fixating effect of 30% acacia is evanescent, whereas that of agar is longer and that of mineral oil seems permanent.

Western New York Section.

University of Rochester School of Medicine, February 13, 1932.

6048

Immunological Identity of Soy and Jack Bean Urease.

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Since proteins are usually species specific it might be expected *a priori* that soy urease would be chemically distinct from jack bean urease. In order to decide this question we have treated clear solutions of soy urease with jack bean antiurease. The antiurease was obtained by injecting crystalline urease into rabbits, followed by purification of the antiurease according to our method.[1] In all cases a precipitin reaction was obtained even when the soy urease contained as little as 1 unit per cc. Furthermore, we have found that soy urease is inactivated by the addition of jack bean antiurease. This inactivation is not so great as the inactivation of jack bean urease by jack bean antiurease. However, the difference is probably due to the presence of colloidal impurities in the soy urease. Table I shows some of our results.

TABLE I.

	Urease Units Recovered
1 cc. soy urease	1.20
1 cc. soy urease + 0.1 cc. jack bean antiurease	0.76
1 cc. jack bean urease	1.18
1 cc. jack bean urease + 0.1 cc. jack bean antiurease	0.42

As further confirmation of the inhibitory effect of jack bean antiurease on soy bean urease we have employed animal tests. Soy urease is as toxic to rabbits as is jack bean urease. If soy urease were distinct from jack bean urease one could not expect that injection of jack bean antiurease would confer passive immunity against soy urease. We have made tests, using 4 rabbits. Two rabbits

[1] Sumner, J. B., and Kirk, J. S., *Z. f. Physiol. Chem.*, in press.

were first given 80 jack bean antiurease units into the ear vein. Then all 4 rabbits were given 50 units of soy urease intraperitoneally. The 2 unprotected rabbits died within 5 hours. The 2 protected rabbits were not affected.

6049

Sex of Parabiotic Twins in Amblystoma Maculatum.*

R. R. HUMPHREY.

From the Department of Anatomy, School of Medicine, University of Buffalo.

After orthotopic transplantation of the gonadic primordia of *Amblystoma maculatum* (Shaw) the writer[1] found a differentiation of the gonad according to the sex of the donor rather than the sex of the host. When an ovary and a testis developed together in any host, however, the testis, whether that of the graft or that of the host, usually induced a modification of the ovary, reducing it in extreme cases to a rudimentary structure containing relatively few germ cells (freemartin ovary). These results were in marked contrast to those of Burns[2] who found only unisexual (♂♂ or ♀♀) combinations in 80 pairs of animals of the same species which had been joined in parabiosis in embryonic stages comparable to those used by the writer for transplantation of gonadic primordia. To determine whether the local (Buffalo) strain of *A. maculatum* would yield similar results under the conditions of parabiosis, 60 pairs were joined in the spring of 1931. Since the operations were performed late in the season on embryos of rather low viability, only 16 pairs survived to ages of 50 days or over. In 15 of these the gonads of both members were sufficiently differentiated to permit positive identification of sex. The combinations of sexes in these pairs were found to be as follows: 1 ♀♀; 6♀♂; 8 ♂♂. In all 6 heterosexual pairs the ovaries of the female member had undergone modification under the influence of the male twin. The central ovarian cavity was absent and the cortex reduced, though in no case to a completely sterile condition. In 2 cases the development of hilar and medullary germ cells in one ovary of the female was

* This study has been aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Humphrey, R. R., *J. Exp. Zool.*, 1929, **53**, 171.

[2] Burns, R. K., Jr., *J. Exp. Zool.*, 1925, **42**, 31.

sufficient to give that gonad the character of a retarded testis; the other ovary in each of these animals, however, exhibited only the usual freemartin state with little or no evidence of reversal. In the 8 pairs classed as ♂♂ the gonads of both members were in every case normal testes, showing no evidence of a reversal from ovaries. The apparent excess of ♂♂ pairs and the occurrence of but one ♀♀ pair are therefore regarded as a chance result, due to the small number of pairs surviving.

The occurrence of heterosexual pairs in the above experiment and their reported absence in the 80 pairs studied by Burns at first suggested to the writer a possible hereditary difference in sex-determining factors between the 2 strains of *A. maculatum* used (Buffalo strain, New Haven strain). This interpretation, however, was rendered untenable by the results obtained by Witschi, Gilbert and Andrew,[3] who repeated Burns's experiments with embryos of the same strain (New Haven), and reported the occurrence of 18 heterosexual combinations among the 41 pairs examined. Since the modified ovaries of heterosexual pairs in the writer's material (and presumably those in the material of Witschi and his coworkers also) are usually very strikingly different from both the normal ovary and the normal testis, it seemed improbable that such gonads could have been overlooked or misinterpreted by Burns. The writer accordingly requested of Dr. Burns the opportunity to examine his material in order to determine if possible the reason for the apparent contradiction of his findings by the results obtained by other investigators. To this request Dr. Burns very kindly assented, and placed at the writer's disposal a large part of the material on which his report was based. Unfortunately a number of slides of this material had been lost through breakage, but since the pairs of animals thus unaccounted for in my examination lie in but a limited age-range in one series, their loss can not be regarded as affecting the validity of any conclusions from the remaining material, which included 57 of the original 80 pairs described by Burns. Among these are *all* of the 24 pairs kept to ages of 5 months or more.

Microscopic examination of this material forces one to the conclusion that reversal of sex has already occurred or is in progress (in 8 cases) in one member of all pairs primarily heterosexual. *The oldest and best differentiated pairs are invariably unisexual.* If the pair is ♀♀, the ovaries of neither member ordinarily show any

[3] Witschi, E., Gilbert, W., and Andrew, G. O., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 278.

morphological evidence that reversal has occurred. A small number of the younger or less differentiated pairs classed by Burns as ♂♂ are, however, apparently of heterosexual derivation. In these, as a rule, the male member appears dominant, while the female member is either essentially indifferent, or, if more advanced in development, shows a varying degree of reversal of the ovaries to testes. The majority, in fact, possess a testicular structure in such large parts of their gonads that these can only be classified as testes. Their derivation from potential ovaries is indicated by the occurrence of sterile regions, or regions in which the germ cells are chiefly cortical in position. None of these gonads is a typical freemartin ovary, however, and in none is an ovarian cortex at all prominent. This marked suppression of ovarian features, together with the characteristic testicular structure of large portions of these gonads, naturally led to their classification by Burns as retarded testes.

In only one pair was there found any indication of the reversal of a male to a female. In this pair the gonads of one member are ovaries, either modified or in a very early stage of differentiation. Germ cells are few, but are chiefly cortical in position and some are in oöcyte stages. No ovarian cavity is present. The 2 gonads of the supposed male member of this pair are quite unlike in structure. One resembles an early testis, much retarded, with its few germ cells chiefly in a central location; the other is more like an early ovary, since its germ cells are chiefly in a cortical position, and a central cavity is lacking. Aside from this pair, which is scarcely past the indifferent stage, no pair gives evidence of reversal of a male member to a female, though the proportion of ♀♀ pairs suggests the probable occurrence of such a reversal.

The ratio of 36 ♀♀ to 44 ♂♂ originally reported by Burns was interpreted by him as a 1 :1 ratio. Burns admitted the probability that some pairs classed by him as ♂♂ might actually be indifferent, but called attention to the fact that their reclassification as such would tend merely to cause a closer approximation to the 1 :1 ratio. In the 57 pairs of Burns's animals examined, the writer has found 6 pairs classed by Burns as ♂♂ which might preferably be classed as undifferentiated. The sex distribution of the remaining pairs may be stated as 26 ♀♀ : 8 ♀♂ : 17 ♂♂. This is far from the ratio of 1 ♀♀ : 2 ♀♂ : 1 ♂♂ required on the assumption that no reversal of sex has occurred in any pair. But since 7 of the 8 pairs classed as heterosexual are either essentially ♂♂ in character or show the male member apparently dominant, and since older pairs are invariably unisexual, it is probable that eventually these animals could only have

been classed as 27 ♀♀ pairs and 24 ♂♂. This is a close approach to the 1:1 ratio expected if reversal of heterosexual pairs should occur without a marked prepotency favoring either sex.

In view of the histological findings and sex ratios stated above, it seems logical to conclude that reversal of one member of each heterosexual pair has either occurred or was in progress when the animals were killed. Naturally the question arises as to why this should have occurred in Burns's animals and not in those of the writer or of Witschi and his co-workers. The answer to this, I believe, must be sought in the conditions under which these animals were reared.

The most striking feature of Burns's parabiotic twins is their marked retardation in development and differentiation. This in some cases, perhaps, is due to the handicap imposed by a very complete or intimate union of the 2 members. Burns[4] states that these animals were fed chiefly on Daphnia and small ostracods. Either from inability to capture sufficient numbers of these small organisms or due to distaste for them, the parabiotic animals remained undernourished, as evidenced by the entire absence of fat from the fat bodies in practically all specimens. Normal well-fed larvae of *A. maculatum* of the New Haven strain, according to Twitty and Schwind,[5] metamorphose at about 70 days after operation (at Harrison's Stages 26-31) and at that time range from 48 to 55 mm. in length. Burns's animals usually failed to metamorphose until 5 or 6 months of age and measured only 25 to 30 mm. in total length at that time. As a rule sex is readily distinguished by microscopic examination in normal *A. maculatum* larvae of 50 days. In Burns's animals the indifferent period is prolonged 30 days or more beyond this time, and some animals at 5 months of age are still difficult to classify as to sex.

Malnutrition even in unoperated larvae of *Amblystoma maculatum* prevents the normal increase in germ cells in the gonads, and causes them to remain for a longer time in the indifferent state. Such retarded gonads, when subjected to the influence of the gonads of a parabiotic twin, probably undergo reversal more readily and completely than would the rapidly differentiating gonads of a well nourished animal. In other words, sex-differentiation is probably subject to various modifying influences, and the influence of a parabiotic partner of another sex, superimposed on retarded development due to malnutrition, may differ markedly from the effect of the

[4] Personal communication.

[5] Twitty, V. C., and Schwind, J. L., *J. Exp. Zool.*, 1931, **59**, 61.

parabiotic twin alone. The effects of dietary and other factors on sex-differentiation in unoperated *Amblystoma maculatum* require further investigation in view of Burns's results and of the abnormal sex ratios reported by the writer[6] in laboratory-reared animals of this species.

6050

Influence of Posterior Pituitary Extracts on Mineral and Water Exchange in Children.*

R. C. MANCHESTER. (Introduced by S. W. Clausen.)

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The influence of posterior pituitary extracts on mineral and water exchange was studied in 2 boys aged 9 and 5 years. The environmental and metabolic conditions, except for changes induced during experimental periods, were kept as constant as possible throughout the study. Each day was divided into 4 six-hour periods beginning at 6:00 A.M. At the beginning of each period, the subject voided, was weighed, and then given an accurately prepared meal of known weight and composition. The 4 meals of the day were identical in every respect and were prepared from simple foods of relatively constant mineral content. Water balances were calculated by the method suggested by Newburgh, Johnston and Falcon-Lesses.[1] On ashed urine sodium was determined by the uranyl acetate method of Barber and Kolthoff[2] and potassium by the chloroplastinate method of Shohl and Bennett.[3] In view of the short periods utilized, mineral excretion in the stool was disregarded and average urinary excretion on control periods was used as a base line for determining fluctuations from normal during experimental periods. The posterior pituitary preparation pitressin was administered in all instances by the subcutaneous route.

The results of the 2 experiments presented in Table I clearly dem-

[6] Humphrey, R. R., *Anat. Rec.*, 1931, **48**, 22.

* This investigation was aided by a grant from the Research Fund of the Rockefeller Foundation.

[1] Newburgh, L. H., Johnston, M. W., Falcon-Lesses, M., *J. Clin. Invest.*, 1930, **8**, 161.

[2] Barber, H. H., Kolthoff, I. M., *J. Am. Chem. Soc.*, 1928, **50**, 1625.

[3] Shohl, A. T., Bennett, H. B., *J. Biol. Chem.*, 1928, **78**, 643.

TABLE I.
Effect of Pitressin on Water Balance and Urinary Sodium Potassium and Chlorid Excretion. H. D., 5 yrs.

Days	Time	Body* Weight	Pitressin	Total Water Available gm.	Total Water Lost gm.	Total Water Balance gm.	Urine Na m.eq.	Urine K m.eq.	Urine Cl m.eq.
			PART 1.						
1	6 A.M.—6 A.M.	20410		1489	1632	—143	33.7	29.0	47.0
2	6 A.M.—6 A.M.	20325		1488	1614	—126	32.0	30.0	46.0
3	6 A.M.—6 A.M.	20260	0.4 cc./3 hr.	1989	1530	+459	66.0	23.0	75.0
4	6 A.M.—6 A.M.	20795		1489	2206	—717	19.0	24.0	31.0
5	6 A.M.—6 A.M.	20150		1489	1386	+103	10.7	26.0	26.0
6	6 A.M.—6 A.M.	20345		1487	1409	+ 68	26.0	26.0	42.0
7	6 A.M.—6 A.M.	20500		1488	1615	—127	36.0	26.0	48.0
			PART 2.						
	6 A.M.—12 N.	20050		373	375	— 2	10.0	12.5	18.0
	12 N. —6 P.M.	20070		372	484	—102	9.9	10.7	14.9
	6 P.M.—12 Mn.	19975		370	314	+ 56	3.7	4.8	8.0
	12 Mn.—6 A.M.	20060		379	293	+ 76	2.5	4.7	6.4
1	6 A.M.—6 A.M.			1486	1466	+ 20	26.1	32.7	47.3
	6 A.M.—12 N.	20165	0.3 cc./3 hr.	625	344	+281	19.0	12.7	25.0
	12 N.—3 P.M.	20465		355	291	+ 64	14.0	4.3	13.3
	3 P.M.—6 P.M.	20570		19	588	—569	17.6	4.5	10.5
	6 P.M.—12 Mn.	19950		370	446	— 76	3.0	7.2	6.2
	12 Mn.—6 A.M.	19905		369	303	+ 66	1.7	4.7	3.5
2	6 A.M—6 A.M.			1738	1982	—244	55.3	33.4	58.5
3	6 A.M.—6 A.M.	20000		1488	1431	+ 57	11.2	29.5	28.5
4	6 A.M.—6 A.M.	20135		1489	1505	— 16	34.6	34.0	50.0
Average excretion on 8 control days							32.0	31.0	46.0

* Body weight at the beginning of each period.

onstrate that pitressin has a pronounced effect on mineral as well as water exchange.

The retention of water during the period of antidiuresis is associated with a large increase in urinary sodium and chlorid excretion. Potassium output is diminished in one experiment but is unaltered in 2 other studies. The effect of pitressin lasts about 3 hours after the last injection and is followed by an enormous water loss, exceeding the storage of the antidiuretic period by several hundred grams. The increased sodium and chlorid excretion persists throughout the period of diuresis. During the recovery period, which lasts over several days, appreciable amounts of sodium and chlorid are retained.

The storage of water associated with a loss of body sodium and chlorid under the influence of pitressin is of particular interest in view of the predominating belief that an accumulation of water in the body entails a retention of base. In day 3, part 1 of the table, the body loses 34 m.eq. of sodium and 29 m.eq. of chlorid simultaneously with the storage of 459 gm. of water. If the assumption

is made that the greater part of the water is retained as extracellular fluid, which under normal conditions contains about 15.7 m.eq. of sodium per hundred grams, a total sodium deficit of approximately 100 m.eq. exists. The excessive water loss during the period of diuresis more than compensates for the sodium and chlorid deficit and may represent an attempt on the part of the organism to restore the normal electrolyte composition of the body fluids.

A significant diurnal variation in mineral excretion is noted in day 1, part 2 of the table. It has been noted consistently throughout a long series of unpublished experiments and is independent of diurnal variations in water excretion and metabolic rate.

Pacific Coast Section.

University of California School of Medicine, February 17, 1932.

6051

The "Multiple Partition Coefficient" Hypothesis in Relation to Permeability.

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Irwin's "multiple partition coefficient" theory[1] has been examined with reference to the adequacy of its theoretical and experimental bases. This theory proposes that the rate of diffusion of a substance from (A)/ to (C) in a system: aqueous phase (A)/ non-aqueous phase (B)/ aqueous phase (C) depends upon the partition coefficients between adjacent phases. Suppose the diffusing substance to be a dye originally dissolved in (A): with time its fugacity in (B) will approach but not exceed that in (A) (which we may assume to be kept constant). Its stoichiometric concentration in (B) will assume a value which characterizes the partition coefficient, but its fugacity from (B) will be independent of the partition coefficient. The rate of diffusion out of (B) into (C) will depend upon both its fugacity and its stoichiometric concentration in (B), and also of course in (C). The rate of diffusion across either or both phase boundaries may also be affected by local conditions at the interface peculiar to the nature of the 2 phases and the diffusing substance. For these reasons the hypothesis, insofar as it is based on partition coefficients alone, is physically unjustifiable, and we would expect it to have a very limited applicability.

The supposed experimental proof[1] of the hypothesis rests upon parallelism between the relative rates of uptake of a relatively small number of dyes into the sap of living cells of *Valonia* or *Nitella*, and their uptake by the "sap" of an artificial cell. This artificial cell consists of a horizontal glass tube bearing 3 upright arms; the horizontal tube is filled with $CHCl_3$, which separates sea water in one

[1] Irwin, M., Proc. Soc. Exp. Biol. and Med., 1928, **26**, 125.

end arm from natural or artificial sap in the other. Sea water, $CHCl_3$, and sap correspond to (A), (B), and (C) above, and the $CHCl_3$ is supposed to correspond to the plasma membrane of a living cell. All are stirred. Dyes are placed in the sea water and their relative rates of entry into both (B) and (C) noted.

As indicated above, the time-distribution relations of the dye in the artificial cell are not determined by partition coefficients alone. Furthermore, since partition coefficients were not determined, the artificial cell affords no evidence at all as to the part played by them. It is not therefore allowable to conclude that partition coefficients account for the general parallelism between artificial and living cells. Such parallelism is better correlated with other well-defined characteristics of the dyes used. No dye with a formula weight exceeding 456, nor any acid dye, was found in the sap of either the living or artificial cell at the time of the only recorded observation (3 hours). Among the basic dyes, the most highly ionized penetrated slowest; and all the acid dyes tested are strong acids. Formula weight, ionization, or sign of charge might account for the failure to enter the cells.

Confirmation of the above criticisms was obtained by applying similar tests to a series of redox indicators and other dyes: we used 4 indophenols, 3 indigo sulphonates, methylene blue, erythrosine, and brilliant cresyl blue. Many discrepancies between the artificial and living cells were found. In particular, all the indophenols went into the $CHCl_3$ of the artificial cell; all except one entered living *Valonia* cells, but none went into the "sap" of the artificial cell. The multiple partition coefficient hypothesis therefore, rests upon unsound theoretical and experimental bases.

6052

Effect of Liver Poisoning on the Action of Parathyroid Extract.

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The mechanism by which parathyroid extracts increase the level of the blood calcium is almost completely unknown. Sendroy and Hastings[1] have shown that *in vitro* such extracts have no effect on

[1] Sendroy, J., and Hastings, A. B., *J. Biol. Chem.*, 1927, **71**, 783, 797.

the solubility of slightly soluble calcium salts. Similarly, we found that parathyroid extract did not increase the calcium content of serum incubated with fresh bone preparations. Bodansky, Blair and Jaffe[2] have shown that in the living animal the increase of the blood calcium on prolonged treatment is brought about by dissolution of the bone salts.

Since the effect of the extracts does not seem to be on the bone directly, the idea suggests itself that some other organ or tissue may be also involved in the action of the parathyroid hormone. Because the liver carries on so many of the chemical functions of the body, it was decided to first make some attempts to test whether the liver might be involved in the action of parathyroid extracts. The choice of the liver was made more attractive by the findings of Minot and Cutler[3] that calcium reserve is a protection against acute carbon tetrachloride and chloroform poisoning.

We determined the response of dogs with experimentally induced liver injury, to parathormone injection.* To date only phosphorus has been employed to produce liver damage, and in these animals it has been found that the normal response to parathormone disappears.

A typical protocol of the results obtained with phosphorus poisoning is the following:

Dog B. Weight 11 kilos. Normal Control Period. Date, 12-10-1931.
100 units of parathormone injected subcutaneously. Blood drawn immediately and at 6 and 24.5 hours after the injection.

		Analyses.			
Time after injection, hours		0	6	24.5	
Calcium mg./100 cc.		11.7	14.3	17.3	Inorganic serum phosphate in control sample, 5.0 mg. per 100 cc.

First Phosphorus Period.

12-15-1931	2:30 P. M.	5 mg. P in olive oil injected subcutaneously
12-16-1931	10:00 A. M.	6 mg. P in olive oil injected subcutaneously

100 units parathormone injected

Analyses of blood samples

Time after injection, hours	0	6.5	24
Calcium mg./100 cc.	12.9	14.7	13.7

Dog recovered and was quite comfortable. Allowed a rest period to 1-14-32.

Second Phosphorus Period

1-14-32	9:30 A. M.	5 mg. P in olive oil injected
1-15-32	9:30 A. M.	8 mg. P in olive oil injected

100 units of parathormone injected

Analyses of Blood Samples

Time after injection, hours	0	7	11.5	24	77
Inorganic serum phosphate mg./100 cc.	5.05	4.60	4.05	2.95	2.45
Calcium mg./100 cc.	11.40	11.85	11.70	11.65	9.50

[2] Bodansky, A., Blair, J. E., and Jaffe, H. L., *J. Biol. Chem.*, 1930, **88**, 629.

[3] Minot, A. S., and Cutler, J. T., *J. Clin. Invest.*, 1928, **6**, 369.

* The parathormone used in these experiments was kindly furnished us by Eli Lilly and Company.

1-18-32 2:30 P.M. The dog sacrificed 77 hours after last injection period. A blood sample was taken at this time with the results given in last column. The serum of this blood sample was highly jaundiced.

Autopsy. The liver was of a pale brownish yellow color, very soft and friable with all the characteristic signs of phosphorus poisoning. Kidneys seemed normal.

These results necessarily must be taken with caution but they do seem to point to a connection between the liver and parathyroid action. The effect of other liver poisons is now being studied.

6053

Studies on Arginine II. Phosphoarginine as a Possible Precursor of Creatine.

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Arginine was first suggested as the mother substance of creatine by Czernecki.[1] From theoretical considerations the hypothesis has been generally regarded as an attractive one. It is in accordance with the fact that arginine, creatine and creatinine are the most abundant of the guanidine derivatives present in the animal organism. It is possible to postulate a series of plausible reactions by which the conversion of arginine to creatine might conceivably be accomplished.[1] It is also in harmony with the mutually exclusive occurrence of arginine and creatine as demonstrated by Kutscher and Ackermann.[2] These investigations have shown that creatine, a characteristic constituent of vertebrate muscle, is replaced by arginine in invertebrate muscle. Indeed the corresponding phospho esters, in which the muscle arginine and creatine largely occur, are even functionally equivalent.[3, 4]

Nevertheless, efforts to demonstrate the origin of creatine from arginine have been unsuccessful, almost without exception. Numerous investigations, described in Hunter's monograph,[5] and others referred to by Hyde and Rose[6] have been entirely negative in result or open to serious criticism upon some crucial point.

1 Czernecki, W., *Z. physiol. Chem.*, 1905, **44**, 294.

2 Kutscher, F., and Ackermann, D., *Z. Biol.*, 1926, **84**, 181.

3 Meyerhof, O., and Lohmann, K., *Naturwissenschaften*, 1928, **16**, 47.

4 Lundsgaard, E., *Biochem. Z.*, 1930, **230**, 10.

5 Hunter, A., *"Creatine and Creatinine,"* Longmans Green, 1927.

6 Hyde, E. C., and Rose, W. C., *J. Biol. Chem.*, 1929, **84**, 535.

Doubtless one of the greatest difficulties in experiments *in vivo* has been the intervention of arginase, as a result of which administered arginine suffers rapid conversion to ornithine and urea. This competitive mechanism leaves little if any arginine available for the various reactions which are alleged to lead to creatine formation.

With this difficulty in mind, we have studied the possible rôle of phosphoarginine as a precursor of creatine. Unlike arginine, phosphoarginine is not acted upon by arginase.[7] In consequence, the arginase mechanism fails to enter as a disturbing factor and there would be, seemingly, every opportunity for phosphoarginine to undergo conversion to phosphocreatine if arginine and creatine are actually related in accordance with the hypothesis mentioned.

Phosphoarginine was prepared by the method of Meyerhof and Lohmann.[7] The large, edible crab *(Cancer magister)* was used as the source. The animals were chilled thoroughly before killing. The legs were rapidly removed, minced by the use of a chilled grinder, and the mincings immediately frozen with liquid air. The frozen material was then triturated with cold trichloroacetic acid and the extract submitted to the barium precipitations recommended by Meyerhof and Lohmann. After one or 2 unsuccessful preliminary attempts at isolation, we found it desirable to omit the final treatment with sulphuric acid and subsequent reprecipitation of the barium salt, since the desired material was being lost, presumably by hydrolysis in the acid solution. In the first successful preparation 175 mg. of the soluble barium salt were obtained from 3 crabs. Two mg. were used for the determination of barium and phosphorus and 3 mg. for arginine. The preparation was found to contain 20% Ba, 9.8% P, and 57% of arginine (theoretical values for pure barium salt of phosphoarginine are Ba, 21.3; P, 9.64; arginine, 54.1). All of the arginine and phosphorus present in the material were in the combined form. The analysis was conducted after preliminary acid hydrolysis in $2N$ H_2SO_4. Barium was determined gravimetrically as $BaSO_4$, and phosphorus (in the filtrate) by the method of Fiske and Subbarow.[8] Arginine was determined in a separate portion of hydrolysate by the method of Kiech, Luck, and Smith.[9]

A second preparation of the salt from 4 crabs yielded 480 mg. of less pure material (35% barium salt of phosphoarginine).

In order to conserve the material available and to extend its use

[7] Meyerhof, O., and Lohmann, K., *Biochem. Z.*, 1928, **196**, 49.

[8] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

[9] Kiech, V. C., Luck, J. M., and Smith, A. E., *J. Biol. Chem.*, 1931, **90**, 677.

over as many animals as possible, mice were used as experimental subjects. For at least 3 weeks prior to experiment they received a standard diet of ground cereal grains, whole milk powder, alfalfa meal, bone meal, yeast, sodium chloride, and cod liver oil. For the 24 hours preceding analysis the animals received water only. On withdrawal of the food each animal was placed upon a coarse wire screen over a lightly-paraffined iron mortar, covered with an inverted funnel. Eighty mg. of the barium salt in 1.0 cc of water were treated with an equivalent amount of sodium sulphate (to remove the barium) and diluted to 1.5 cc. The suspension was injected subcutaneously in 3 portions of 0.5 cc. each at 4 hour intervals, the first injection being at the time of food withdrawal. Controls were treated in identical fashion, except that 1.0 cc. of barium chloride was used in place of arginine barium phosphate. Under these conditions the mechanical loss of phosphoarginine was avoided. Barium was also removed without acidification and hence without danger of phosphoarginine hydrolysis.

During the experimental period the animals were kept in a warm room at approximately 28°. Twenty-four hours after the first injection the animals were stunned, then frozen and ground with liquid air in the mortar containing the excreta of the 24-hour period. After being thoroughly powdered, aliquot portions were weighed out and used in the determination of urea (method of Allen and Luck[10]) and total creatinine. For the latter, 4 different methods were tried but only that of Rose, Helmer, and Chanutin[11] was found

TABLE I.

Control Mice				Experimental Mice			
Mouse No.	Wt.	Total Creatinine	Urea	Mouse No.	Wt.	Total Creatinine	Urea
	gm.	mg. per 100 g.	mg. per 100 g.		gm.	mg. per 100 g.	mg. per 100 g.
10	28	171.2	153.6	23	23	138.7	——
11	25	152.6	133.0	24	23	141.0	152.5
12	24	166.3	151.9	25	23	135.6	161.8
13	26	178.9	132.2	26	21	172.3	108.2
14	26	139.8	133.3	27	28	149.0	126.9
15	22	131.9	164.0	28	25	140.6	——
16	22	140.0	135.9	29	26	145.7	143.6
17	21	133.6	150.6				
18	25	144.1	124.6				
19	24	139.6	——				
20	24	143.5	——				
21	23	142.1	152.5				
22	22	145.6	155.3				

Mice Nos. 23, 24 received preparation I of phosphoarginine.
Mice Nos. 25 to 29 received preparation II of phosphoarginine.

[10] Allen, F. W., and Luck, J. M., *J. Biol. Chem.*, 1929, **82**, 693.

[11] Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

to give consistent results and close agreement between duplicate determinations. The amount of phosphoarginine (preparation I) injected was sufficient, if totally converted, to yield 1.2 mg. of creatine (as creatinine) per gram of mouse. This would represent an increase of 90% over the basal creatine-creatinine level. Any appreciable hydrolysis of the phosphoarginine would also be reflected by significant increases in urea, because of the rapid metabolism of arginine. With preparation II of phosphoarginine, 30% increases in total creatinine would be the maximum attainable.

The results presented in Table I demonstrate that under the conditions of these experiments phosphoarginine failed to undergo conversion into phosphocreatine, creatine or creatinine. They do not support the hypothesis that arginine or phosphoarginine is the mother-substance of creatine.

6054

Influence of Digitalis on the Sensitivity of the Cardiac Vagus Endings.

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The work of Heymans[1, 2] on the mechanism of the bradycardia of digitalis seems to dispose of the older theory that this drug stimulates the vagal centers directly. It supports Straub's[3] suggestion that the slowing is due to a direct myocardial action, the sinus pacemaker becoming more responsive to vagal tone. In support of this viewpoint, Rothberger and Winterberg[4] have claimed that digitalis lowers the threshold to electrical stimulation of the vagus trunks, and Weger has observed[5] that it makes the parasympathetic endings in the intestine more irritable to stimulation by pilocarpine. However, Weiss[6] regards the slowing after digitalis as due to reflexes originating in the viscera innervated by the vagus. In order to obtain data regarding the validity of the first of these theories,

[1] Heymans, J. F., and Heymans, C., *J. Pharm. Exp. Therap.*, 1926, **29**, 203.
[2] Heymans, C., *Ergeb. der Physiol.*, 1929, **28**, 300.
[3] Straub, W., *Heffter's Handb. der Exp. Pharmakol.*, **2**, 1422.
[4] Rothberger and Winterberg, *Arch. f. Physiol.*, 1910, **132**, 233.
[5] Weger, P., *C. R. Soc. Biol.*, 1927, **96**, 803.
[6] Weiss, S., *Med. Clinics of N. Amer.*, 1932, **15**, 963.

we have attempted to demonstrate the presence or absence of an altered vagus sensitivity after digitalis, by comparing the responses in pulse rate to parasympathetic stimulants before and after administration of this drug.

The slowing caused by choline or acetyl choline in 6 anesthetized and unanesthetized dogs and cats, including one dog in which the adrenals were removed and the stellate ganglia and upper thoracic sympathetic trunks destroyed, was so slight that these drugs seemed of no value as tests for vagus sensitivity. We, therefore, selected physostigmine as a stimulant of the vagus endings. In 3 dogs, the vagi were cut aseptically, and after a 24-hour recovery period, 0.3 mg. per kilo of physostigmine was injected intravenously and the pulse rate changes recorded. The next day, 20 mg. digitalis per kilo, as the dilution of an active tincture, was given intravenously, and 2 hours later physostigmine was reinjected in the same dosage. The maximum slowing of the control physostigmine-action was 18, 16, and 28% of the control rates. After the digitalis, the slowing was 24, 19, and 14%, respectively, of the rates after the digitalis. There was thus no definite increase in the pulse rate response to the physostigmine after digitalization. The influence of digitalis on the minimum dose of physostigmine required to cause slowing in a vagotomized dog was also studied. Digitalization did not alter this threshold dose.

Both types of experiments failed to provide any definite evidence that digitalis sensitized the vagus endings in the heart to direct parasympathetic stimulation. Therefore, these results lend additional weight to the view that digitalis-bradycardia, like digitalis-emesis,[7] is due to reflexes originating in the viscera.

[7] Hanzlik, P. J., and Wood, D. A., *J. Pharm. Exp. Therap.*, 1929, **37**, 67. Dresbach, M., and Waddell, K. C., *J. Pharm. Exp. Therap.*, 1928, **34**, 43.

6055

Alleged Protective Action of Colloidal Dyes in Anaphylactic Shock.*

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Protection with congo red and trypan blue against anaphylactic shock of guinea pigs has been claimed recently by Nikolaiev and Goldberg.[1] They used 7 sensitized guinea pigs treated with congo red and trypan blue, and 4 sensitized guinea pigs without protective dye-treatment. The dyes were injected intraperitoneally in total doses of from 10 to 50 mg., in from 10 to 30 minutes before injection of the antigen intraperitoneally; the dyes and the antigen were presumably mixed together in the abdomen. Of the 7 dye-treated guinea pigs, 3 showed symptoms of anaphylactic shock and 4 did not; the 4 controls showed symptoms. Such results on so small a group of animals are not impressive. Using a much larger group of animals from 2 species in a study of the same problem, approached from different angles, I obtained negative results 4 years ago and again recently. A record of my results at this time may be useful to others, for avoiding unprofitable experimentation along similar lines.

A total of 70 animals (51 guinea pigs and 19 pigeons) was used. The colloidal dyes tried were: congo red, mercurochrome, rose bengal, and vital red, which were always used in 1% strength in 6% dextrose solution. The following conditions were used with sensitized animals: intravenous injection of the dyes in from 5 to 27 minutes before injection of the antigen; daily intravenous injection for 3 days of the dyes before the antigen; intravenous injection of mixtures of the dyes and antigen, freshly made and after incubations for ½ hour and 1 hour; intravenous injection of mixtures of the dyes and antigen freshly boiled together (the mixtures did not coagulate[2]). In these experiments, the total dosage of the dyes was 40 mg. per kilo, and of the antigen 0.2 cc. (horse serum). Normal animals were injected hypodermically with the following mixtures of the dyes and horse serum to see if the process of sensitization

* Supported, in part, by a grant from the Rockefeller Fluid Research Fund of the School of Medicine, Stanford University.

[1] Nikolaiev and Goldberg, *Z. f. d. ges. exp. Med.*, 1930, **73**, 475.

[2] Hanzlik, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 364.

could be influenced: fresh mixtures; mixtures incubated for ½ hour and 1 hour; boiled mixtures (no coagulation). In some cases, 0.2 cc. of the serum was used with 4 cc. of dye solution per kilo, and in others equal parts of serum and dye solution. At the end of 2 weeks, 0.2 cc. of horse serum as antigen was injected intravenously. At least 3, and sometimes 5, animals were used for each experimental condition. A total of 15 animals (9 guinea pigs and 6 pigeons) served as untreated controls for both groups of experiments.

The results obtained may be stated briefly: 82% of the 51 treated guinea pigs and 100% of the 19 treated pigeons showed the typical signs and symptoms of anaphylactic shock, in variable degree; about one-half of these animals died, the same being true of the controls. All the guinea pigs showed the typical pulmonary distention. Collectively, the vast majority, or 87%, of all the animals used failed to be protected by the colloidal dyes used. Accordingly, an intrinsic cellular reaction (antigen-antibody) was not prevented by colloidal dyes, some of which could prevent the effects of drugs and toxins.[3] This difference points to fundamental differences in the tissue reactivities of proteins and of drugs and toxins, the latter perhaps acting more in the cell exterior (humoral and cell-surface mechanisms), in accordance with certain postulates and evidences of pharmacology.

Conclusions. The colloidal dyes congo red, vital red, rose bengal, and mercurochrome, tried under a variety of conditions, failed to protect the majority of guinea pigs and pigeons against the typical symptoms of anaphylactic shock. These negative results do not support the positive claims of Nikolaiev and Goldberg, who used congo red and trypan blue. It is suggested that the results with these colloidal dyes point to fundamental differences in the tissue reactivities of proteins and of drugs and toxins.

[3] Hanzlik and Butt, *J. Pharm. Exp. Therap.*, 1928, **33**, 260.

6056

Biochemorphic Aspects of Paraldehyde and Certain Acetals.*

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Paraldehyde has many features recommending it as a hypnotic agent. Conversations with Prof. C. W. Muehlberger and the late Prof. A. S. Loevenhart suggested a preliminary survey, at least, of the biochemorphology of paraldehyde and chemically related substances in the effort to determine whether or not there might be promise of finding a compound in the series with properties approaching more closely to those of an ideal clinical soporific. The compounds so far secured have been studied before,[1] but separately and not as a series. Acetal and methylal received clinical trial but were considered unsatisfactory.[2]

In Table I is a list of the compounds we investigated, with their structural formulae, certain physical characteristics, and toxicity data. The latter were obtained by administration to rabbits by stomach tube of dilute water emulsions of the respective substances. Table II is a summary of the comparative depressant action of these aldehyde derivatives as determined in 150 experiments on rabbits, in which observations were made and recorded by methods modified from those described by Magnus.[3]

These related compounds have equal rapidity and duration of action when given in doses of equal effectiveness. They are all excreted by the lungs in considerable amounts. Peripheral vasodilatation and increased heart rate are produced by all of them, but their effect on pupil size is inconstant. As may be seen from the summary data presented in Table II, none of these agents is particularly analgesic in action.

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* Aided in part by the Purington Research Fund.

† Fellow of the National Research Council.

[1] V. Cervello, *Arch. Exp. Pharmakol. u. Path.*, 1882, **16**, 265; V. Mering, *Berlin Klin. Wchnschr.*, 1882, **19**, 648; Langgard, A., *Therap. Monatsh.*, 1886, **24**; Personali, E., *J. de Pharm. et de Chim.*, 5th series, 1887, **15**, 33; Richardson, B. W., *Asclepiad*, 1888, **5**, 135; Dieballa, G., *Arch. Exp. Pharmakol. u. Path.*, 1894, **34**, 137.

[2] Peretti (of Andernach), *Der Irrenfreund*, 1883, **25**, 65.

[3] Magnus, R., *J. Pharm. Exp Therap.*, 1926, **29**, 35.

TABLE I.
Oral Toxicity of Paraldehyde and Certain Acetals in Rabbits.

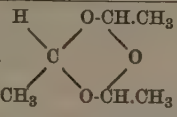
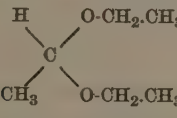
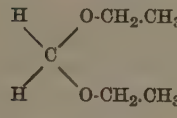
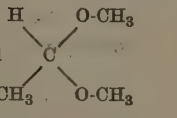
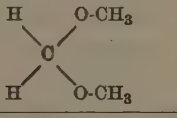
Substance	Formula	Mol. Wt.	Sp. Gr.	Boiling Point	Vol. of H_2O to dissolve one volume	Mortality Ratio millimols per kg.
Paraldehyde		132	0.99	124°C.	8	3/10 at 20 6/10 " 25
Acetal		118	0.82	104°C.	18	0/8 " 25 2/5 " 28.5 5/8 " 30
Ethylal		104	0.85	85°C.	11	0/5 " 15 2/8 " 20 4/8 " 25
Dimethyl-acetal		90	0.86	63°C.	4	0/5 " 45 2/4 " 50 7/8 " 60
Methylal		76	0.86	41°C.	3	1/5 " 60 4/7 " 75

TABLE II.
Summary of Certain Pharmacological Actions of Certain Paraldehyde Derivatives Administered in Dilute Water Emulsion by Stomach Tube to Rabbits.

	Methylal	Dimethyl-acetal	Ethylal	Acetal	Paraldehyde
Average Lethal Dose (LD_{50}) in millimols per kg.	75	50	25	30	25
Average maximum percentage depression normal respiratory rate at ½ average lethal dose	60	10	60	10	70
% of average lethal dose causing complete loss of:					
1. Posture	80	120	80	100	50
2. Progression	60	100	60	67	50
3. Righting reflex	80	120	80	100	50
4. Response to various stimuli	100	120	80	100	90
5. Corneal reflex	100	120	100	100	75

Some conclusions can be drawn concerning the biochemorphic‡ aspects of these substances. Very striking is the difference in the effect of lengthening the carbon chain on the central, and on the terminal carbon atoms. The substitution of a methyl group on the central carbon atom reduces the effectiveness, whether the terminal groups be methyl or ethyl. The change is greater in the case of terminal methyl groups, as methylal and dimethylacetal differ more in effectiveness than do ethylal and acetal. With lengthening of the terminal carbon chain, the transition from methylal to ethylal is accompanied by a greater increase in toxicity than is the transition from dimethylacetal to acetal. However, the increase in hypnotic activity is not the same in the 2 cases, so that while acetal is more effective than dimethylacetal (in equal percentage of lethal dose), methylal and ethylal are about equally effective. The result of an ether linkage transforming acetal into paraldehyde is also striking. While there is an increase in toxicity of only about 20%, there is a much greater increase in hypnotic activity so that paraldehyde is about twice as effective as acetal. These considerations suggest that it would be desirable to investigate the hypnotic action of alpha-trioxymethylene, and the corresponding oxycyclic derivative of ethylal.

Though our findings indicate that none of the compounds studied here equals paraldehyde in value as a hypnotic, these observations may assist in the search for an improvement on it.

‡ The adjective "biochemorphic" has been coined to connote *that pertaining to the relationship between chemical constitution (including physical properties) and pharmacologicl (physiological or biological) action.* Such a term is badly needed especially when the restricted term "chemotherapy" (Ehrlich) is becoming loosely employed to cover this whole field (Dyson, G. M., The Chemistry of Chemotherapy, London, 1928). The noun indicating *the study of the relationship between chemical constitution and biological activity* would be "biochemorphology." The literal meaning of the term, *knowledge of living chemical structure,* really implies the fundamental objective of investigations on the relationships between chemical constitution and biological activity, so that there is no essential conflict with its technical meaning. "Pharmakomorphic" was also suggested for the use indicated (by Dr. T. E. Reynolds), but was not thought to be quite as satisfactory as "biochemorphic".

6057

On the Kinetics of Monoiodoacetate Poisoning in Streptobacterium casei.

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It was previously reported that sodium monoiodoacetate (hereinafter called IA) inhibits lactic production in *Streptobacterium casei* as it does in higher forms.[1] This organism is especially advantageous for a comparative study of lactic acid fermentation, since a single compound, *e. g.*, glucose, will suffice as a source of energy. The glucose supplied is quantitatively converted to lactic acid, and in the absence of nitrogenous compounds—essential for growth—there seems to be no other significant metabolism.[2] Furthermore there is evidence that the course of lactic acid formation by *Streptobacterium casei* is qualitatively the same as in other forms.[3]

The production of lactic acid is a reaction of great importance and of widespread occurrence, being the physiological equivalent of respiration in some forms and its supplement in others.[4] Like oxidation it involves a chain of reactions which are primarily dehydrogenations. The results of our experiments serve to elucidate further some of the properties of the enzymes concerned in lactate fermentation and to indicate still another analogy with biological oxidation.

Lactic acid production was determined by the method of Warburg.[5] The reaction vessels were filled with a gas mixture consisting of 5% carbon dioxide and 95% nitrogen. The required amounts of IA were placed in the sidearms of the vessels and added after a preliminary control period. In the several controls, which received no IA, after a brief induction period the rate of lactic acid formation remained constant for more than 7 hours. In all cases the organisms were suspended in a solution of 2.0% glucose and 0.5% sodium bicarbonate. Little change in pH occurred during an experiment, the lowest final value noted being pH 6.7.

When IA is added to such a system there is a definite "latent

[1] Field, J., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 357.

[2] Kluyver, A. J., and Donker, H. J. L., *Proc. Roy. Acad. Amsterdam*, 1925, **28**, 605.

[3] Virtanen, A. I., *Biochem. Z.*, 1924, **151**, 232.

[4] Kluyver, A. J., The Chemical Activities of Micro-Organisms, London, 1931.

[5] Warburg, O., *Uber den Stoffwechsel der Tumoren*, Berlin, 1926.

period", which is doubtless a function of cell membrane permeability and diffusion rate of IA. This period varies inversely with the concentration of poison, and is followed by a continuous decrease in the rate of lactic acid production. This decrease follows approximately the form of a bimolecular reaction curve of the (integral) form $K = 1/at \cdot x/a-x$. A typical curve is given in Fig. 1. The biological significance of this finding is not clear at present.

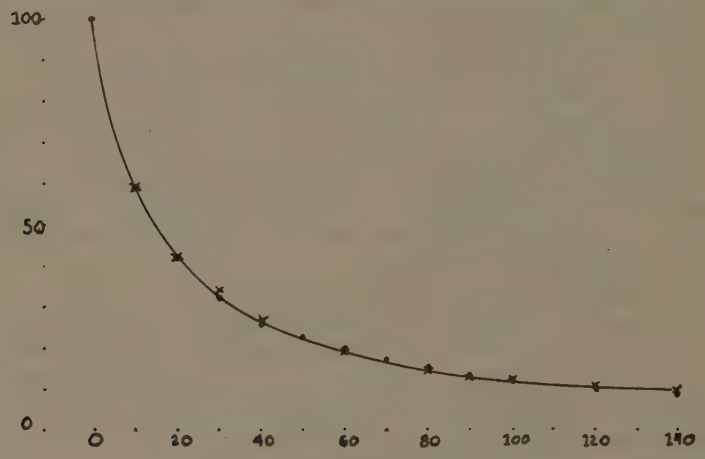

Fig. 1

Curve with 0.02% IA. The average k is 0.0006914. The crosses indicate the course of a curve using k = 0.0006914 for all points, the circles give the experimental curve. The values of k at various times during the experiment are (time in minutes):

t	k	t	k
20	0.0006910	60	0.0006675
30	0.0007080	70	0.0006740
40	0.0007120	80	0.0007080
50	0.0006586	90	0.0007120

Over the whole concentration range studied, varying from 0.0004% to 4.0% IA, and in almost all cases, the rate of lactic acid production in the presence of IA became asymptotic to a finite value. This indicates either that a fraction of the fermentation is stable toward IA or that some other fixed acid is produced in small amounts, or both.

The rate of poisoning was found to be a constant power of the concentration of IA. In this respect the inhibition of lactic acid fermentation by IA is analogous to the inhibition of the respiration of *Aspergillus niger* by copper.[6] "This is the well known power relation which has been derived biologically a number of times . . . .

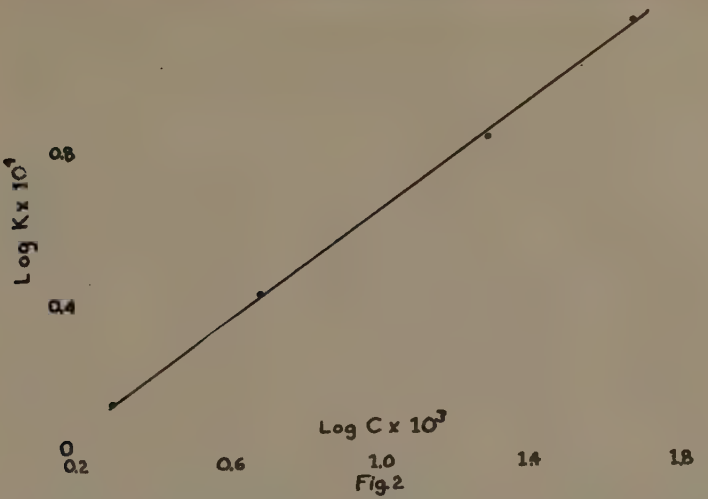

Fig. 2

in work on the effect of toxic substances."[6, 7, 8] This finding is illustrated in Figure 2, which shows a linear relation between the logarithm of the concentration of IA and the logarithm of the velocity constant.

The writers wish to acknowledge the kindness of Dr. C. B. van Niel, of the Hopkins Marine Station, who supplied pure cultures of *Streptobacterium casei* for these experiments.

6058

Influence of Digitalis on the Electrocardiograms of the Chick Embryo.

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Several investigators have recorded electrocardiograms on the embryonic chick, and, in the recent report of Sands,[1] a monophasic tracing was obtained at the 35th hour of incubation. A typical rapid initial ventricular phase and slow final phase, *i. e.*, Q-R-S and T deflections were not obtained until between 50 and 72 hours.

[6] Cook, S. F., *J. Gen. Physiol.*, 1926, **9**, 575.
[7] Chick, H., *J. Hyg.*, 1908, **8**, 92.
[8] Paul, T., Birstein, G., and Reuss, A., *Biochem. Z.*, 1910, **29**, 202, 249.
[1] Sands, J., *Am. J. Physiol.*, 1929, **90**, 496.

It has been determined by Duval and others recently that ingrowth of nerve fibres does not take place until approximately the 6th day, but that the heart generally starts beating at the 10 somite stage or about 29 hours.

We attempted to obtain electrical records of the heart activity, using German silver electrode contacts along an approximate axial lead of the heart. A resistance coupled tube amplifying mechanism, the Nichols-Chase electrocardiograph apparatus, was used for amplifying and recording the action currents in most of the embryos. The Einthoven string galvanometer was occasionally employed on the older preparations.

Digitalis in an aqueous solution, as Digifoline, Ciba, equivalent to 0.1 digitalis leaf per cc. was applied directly to the exposed pulsating heart without changing the electrodes. Variations of temperature were difficult to control, but the surface was kept moist with water approximately at 37°C. Adrenalin and other drugs were applied, but the results of these applications will be reported at a later date.

The records obtained showed a multiphasic curve similar to an adult type electrocardiogram as early as the 35th hour of incubation, an embryo of 12 somites. Digitalis caused a definite change in the form and direction of the final or slow phase of the ventricular deflection. The electrical potential developed was approximately 0.5± millivolts. The occurrence of this multiphasic record, at the earliest period at which an electrocardiogram has been yet obtained, tends to support the opinion of Craib[2] and others that an initial rapid and a final slow phase is the form of electrical response demonstrated by all nerve muscle action currents.

Digitalis produced directional changes of the T waves and partial and complete auriculo-ventricular block both before and after the theoretical time of nerve fibre ingrowth. Auriculo-ventricular block was somewhat more likely to occur, however, after the 5th day.

From these findings it seems apparent that digitalis may act directly on the heart muscle and the conduction system without necessarily autonomic nervous system influence. It is recognized that some changes in form of the electrocardiograms are bound to occur with minor shifts of the heart axis, but we do not believe these shifts great enough to account for the changes which we recorded. It is an interesting observation that the "T" waves of the auricular deflection were unusually prominent in our records.

[2] Craib, W. H., The Electrocardiogram, Brit. Med. Res. Council Pub., London, 1930.

New York Section.

New York Academy of Medicine, March 16, 1932.

6059

Phenomenon of Local Skin Reactivity to Bacterial Filtrates in the Treatment of Mouse Sarcoma 180.

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The phenomenon of local skin reactivity to bacterial filtrates described by one of us (Shwartzman[1]) was later also reproduced in the liver, kidney (Shwartzman[2]), testis, intestines, lymphatic glands, lungs, thymus, guinea pig liposarcoma (Gratia and Linz[3]), stomach (Karsner, Ecker and Jackson[4]), and knee joints (Moritz and Morley[5]). It was elicited with a great variety of microorganisms (Shwartzman[6]) and also with vaccine virus as the preparatory factor (Gratia and Linz[3]). The animals in which the phenomenon was observed were rabbits (Shwartzman[1]), horses, goats (Shwartzman[8]), and guinea pigs (Gratia and Linz[3]). It could not be reproduced in mice and rats (Shwartzman[7]). Assuming that malignant tumors may be of parasitic etiology, Gratia and Linz[3] thought that the hypothetical virus should then be capable of inducing a state of reactivity in the tumor tissue and thus render it susceptible to reacting factors in the blood stream. Five guinea pigs bearing liposarcoma were injected intravenously with *B. coli* culture filtrate. Two guinea pigs which died 24 hours later and 2 killed 48 hours later showed at autopsy hemorrhagic lesions in the tumor tissue and no lesions in other organs. The fifth guinea pig was left alive for

1 Shwartzman, G., *J. Exp. Med.*, 1928, **48**, 247; *J. Inf. Dis.*, 1931, **48**, 339.
2 Shwartzman, G., *J. Exp. Med.*, 1930, **51**, 571.
3 Gratia and Linz, *Comp. Rend. Soc. Biol.*, 1931, Oct. 23.
4 Karsner, Ecker and Jackson, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 319.
5 Moritz and Morley, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 321.
6 Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 207.
7 Shwartzman, G., unpublished observations.
8 Shwartzman, G., *J. Exp. Med.*, in press.

further observations. They selected guinea pigs because of their susceptibility to the phenomenon of local reactivity to bacterial filtrates.

Since it was deemed important to determine whether this phenomenon could be reproduced in transplantable tumors in mice, the effect of bacterial filtrates upon a mouse sarcoma 180 (Crocker Institute) was studied by the present authors. This strain of sarcoma was selected on account of its high growth energy and malignancy. The bacterial filtrate employed was of high phenomenon-producing potency, as previously determined in rabbits (Shwartzman[1]), namely "agar washings" filtrate of meningococcus 44D group I (*i. e.*, filtrate No. 1700 containing 1350 reacting units per cc.). The results can be summarized as follows:

Group I. Nine mice bearing 18-day-old tumors were each injected intravenously with 0.5 cc. of filtrate No. 1700. The injection killed 2 mice within 24 hours. At autopsy there was found extensive hemorrhage in the entire tumor mass and no evidence of it in any other tissue or organ. Twenty-four hours after the intravenous injection the surviving mice showed also extensive hemorrhage in the tumor mass including previously healthy borders. The necrotic mass hardened and separated, leaving a bed of granulation tissue. However, at the borders growth reappeared. Further treatment consisted of intravenous and intraperitoneal injections of 0.5 cc. of the same filtrate 10 and 12 days after the first injection, respectively. Four more mice died from the injections. The surviving mice showed again hemorrhage in the areas of new growth. In one mouse the tumor regressed similarly but began to grow again. In the remaining 2 mice the necrotic mass separated, the granulation tissue filled the bed and complete healing resulted. The mice showed no growth 2 months after tumor inoculation. Their general condition was excellent. One mouse was killed at this time. No growth was discovered in any organ.

Group II a. (4 mice). Mice I and IV received each intravenous injections of 0.25 cc. of filtrate No. 1700 on the 18th, 20th, and 23rd days of tumor growth. Mice II and III received these injections on the 30th and 34th days, in addition. Extensive hemorrhagic necrosis was evident in the tumor masses of all the treated mice within 24 hours after the first injection. The tumor of Mouse I gradually reduced in size until on the 35th day after tumor inoculation there was no tumor left. The healing proceeded uneventfully. Mouse II lost the tumor but died on the 35th day of tumor inoculation. Autopsy showed grossly no tumor growth and no

metastasis. The tumor of Mouse III regressed after the first 3 injections but later increased in size. It died on the 31st day of tumor growth. The tumor of Mouse IV completely disappeared. The mouse appeared completely free of growth on the 35th day of tumor inoculation.

Group II b. (4 mice). Mice V-VIII received each intraperitoneal injections of 0.25 cc. of filtrate No. 1700 on the 18th day of tumor growth. Mice VII and VIII died in 14 and 48 hours after the injection, respectively. The autopsy showed extensive hemorrhagic necrosis of the tumors, and no lesions in other organs. Mice V and VI which showed a similar picture 24 hours after the injection later developed large tumors.

Group II c. (4 mice). Mice IX-XII received each intravenous injections of 0.5 cc. of filtrate No. 1700 on the 18th, 20th, and 23rd days of tumor growth. Mice IX, X, and XII received in addition intravenous injections of the same dose on the 30th and 34th days of tumor inoculation. Here again, the first injection elicited severe hemorrhage in 24 hours. Finally, Mouse IX showed complete regression which was followed by scab formation. There was, however, a doubtful growth observed at the borders on the 37th day of tumor inoculation. The tumor of Mouse X showed a gradual regression to about 1/10 of the growth present before the first injection. After the 4th injection the growth reappeared. Mice XI and XII lost their tumors completely on the 4th week of tumor inoculation and no growth was evident later.

Group II d. (4 mice). Mice XIII-XVI received intraperitoneal injections of 0.5 cc. of filtrate No. 1700 on the 18th day of tumor growth. Mouse XIV died 24 hours later. It showed extensive hemorrhage in the tumor mass. Mice XIII and XV received in addition similar injections on the 20th and 23rd days and Mouse XVI on the 20th, 23rd, and 30th days of tumor growth. The tumor of Mouse XIII regressed to about 1/8 of the size before the first injection. The third injection killed it. The autopsy showed a large necrotic mass with doubtfully active growth. Mouse XV, dead on the 37th day of tumor inoculation, was found at autopsy free of growth. The tumor of Mouse XVI regressed to 1/6 of the size before the first injection and reappeared later.

Group III. In this group 15 mice bearing 17-day-old tumors were each injected intravenously with 0.5 cc. of filtrate No. 1700. Three mice died 24 hours later. They showed hemorrhagic necrosis of the tumors. Within the following 2 weeks there was observed complete regression of the tumor growth in 10 mice. Three of these

mice appeared completely free of tumors on the third week, while in 7 of these mice the growth began again. In 2 mice the growth was uninfluenced at any time after injection.

Control Groups. Nineteen mice in all were set aside as controls, a few for each experimental group. They all developed large tumors, which showed no spontaneous regression at any time. Two of these mice were injected intravenously each with 0.5 cc. of 2% glucose broth containing 0.4% phenol. There was no effect on the development of the tumor observed.

The number of mice employed is too small as yet to allow any conclusions as to the percentage of temporary or complete regressions obtained. However, inasmuch as the tumor strain employed is of high growth energy and malignancy and only very rarely regresses spontaneously, the following can be concluded:

It is possible to elicit prompt hemorrhage in the tumor tissue of mouse sarcoma No. 180 via the blood stream by means of bacterial "agar washings" filtrates (*i. e.,* meningococcus) of high potency in the phenomenon of local skin reactivity to bacterial filtrates.

The first appearance of the effect described resembles very closely the latter phenomenon. The hemorrhage leads to progressive damage of the tumor, which may be followed either by its complete elimination and healing, or by striking regression with further slow reappearance of tumor growth. The individual variations are probably due to spontaneous and active acquired immunity to the reacting factors observed in the phenomenon under discussion (Shwartzman[8]). The effect on tumors appears to be selective, inasmuch as the intravenous and intraperitoneal injections of the filtrates produce no hemorrhagic lesions in other organs of the mouse.

The injections are toxic and kill a large number of mice. Once recovered from the immediate effect, they remain in a good general condition. The repeated intravenous injections of 0.5 cc. of the filtrates employed were most destructive for the tumors; next in effectiveness were repeated intravenous injections of 0.25 cc. and repeated intraperitoneal injections of 0.5 cc. of the filtrates. Repeated intraperitoneal injections of 0.25 cc. remained without definite effect.

This report is of a preliminary nature because of insufficient number of mice and lack of histological studies.

Although there appears to be a close resemblance between the reaction described and the phenomenon of local skin reactivity to bacterial filtrates, it does not necessarily mean that a virus must be responsible for the state of reactivity of the tumor cells to bacterial

filtrates introduced via the blood stream. Studies on other possible explanations are under way.

Torrey and Kahn[9] found that filtrates of certain gram positive anaerobes remained without effect upon transplantable tumors of mice and rats. Inasmuch as their purpose was to employ preparations of high proteolytic activities but the phenomenon-producing potency was not determined, the negative results reported by them are not considered contradictory.

The observations reported in this paper are considered of interest because there appears to be a remarkable selective destruction of a tumor of high malignancy and of rapid growth; and also because being obtained in mice they offer an opportunity for further thorough studies on the relation of the "phenomenon of local skin reactivity to bacterial filtrates" to problems of tumor growth.

6060

B. coli Bacteriophage in the Treatment of B. coli Peritonitis in Mice.

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The bacteriophage which was used for this study was found to produce a complete lysis of 2 virulent strains of *B. coli* which we obtained from patients with peritonitis. One one hundred billionth of a cc. of this bacteriophage caused lysis of approximately one billion bacteria of strain "T" in 10 cc. of broth. The minimal lethal dose of the "T" strain of *B. coli* for mice at the time of the experiments was 1-5 million organisms which killed within 5 to 12 hours after injection of an actively growing, 3-hour, 2% dextrose cooked meat medium culture. Fifty million bacteria which constituted 10-50 M.L.D.'s were suspended in 0.5 cc. of broth and inoculated intraperitoneally into a series of mice. 0.5 cc. of bacteriophage was injected simultaneously into 2 of these mice, and into 2 more mice at varying intervals up to 4½ hours after the bacterial inoculation. This approached closely the lethal period for control animals. In the control series plain broth injections were given at the same intervals as phage. The table shows

[9] Torrey and Kahn, *J. Cancer Research*, 1929.

one of these experiments. From this table one can see that 0.5 cc. of bacteriophage protected all of the mice when given up to 3½ hours after bacterial inoculation. Only 50% of mice could be

TABLE I.

No.	Dose	Used for treatment Broth cc.	Used for treatment Bacteriophage cc.	Interval between inoculation and treatment	Result in 20 hr.	Final result*
1	50 m	0.5		Simultan.	D	D
2	"		0.5	"	L & W	S
3	"		0.5	"	L & W	S
4	"	0.5		15 min.	D	D
5	"		0.5	"	Sl. sick	S
6	"		0.5	"	Sl. sick	S
7	"	0.5		30 min.	D	D
8	"		0.5	"	Sl. sick	S
9	"		0.5	"	Sl. sick	S
10	"	0.5		1 hour	D	D
11	"		0.5	"	Sick	S
12	"		0.5	"	Sl. sick	S
13	"	0.5		2 hours	D	D
14	"		0.5	"	Sl. sick	S
15	"		0.5	"	Sl. sick	S
16	"	0.5		3 hours	D	D
17	"		0.5	"	Sl. sick	S
18	"		0.5	"	Sick	S
19	"	0.5		3½ hours	D	D
20	"		0.5	"	Sick	S
21	"		0.5	"	Active	S
22	"	0.5		4 hours	D	D
23	"		0.5	"	D	D
24	"		0.5	"	Sl. sick	S
25	"		0.5	4½ hours	D	D
26	"		0.5	"	D	D
27	"				D	D
28	10 m				D	D
29	2 m				D	D
30	1 m				Sl. sick	S
31			2		L & W	S
32			4		L & W	S

* Control mice begin to die in 5 hours and at least half of them are dead before 7 hours after inoculation.
S = survived. D = died. L & W = living and well.

saved when bacteriophage was given after 4 hours. In similar experiments there was occasional recovery when phage was inoculated after 4½ hours or later. Control animals receiving *B. coli* filtrate instead of phage were not saved. The bacteriophage itself proved to be innocuous, for 2 cc. and 4 cc. of it injected respectively into the peritoneal cavity of 2 mice did not produce any harmful effect. Both mice survived and the one inoculated with the larger dose suffered only slight discomfort for 1 or 2 hours, probably due to distention from the quantity of fluid injected. With smaller doses of phage or

larger doses of bacteria the period after injection of organisms at which protection was obtained with phage, was shortened.

In another series of experiments bacteriophage was given subcutaneously, while the organisms were injected intraperitoneally. In these experiments, only mice inoculated with bacteriophage simultaneously or 15 minutes after the injection of bacteria could be saved. The mice inoculated with bacteriophage at later periods succumbed at the same time as the controls. When mice which had survived these experiments were autopsied several days later no signs of peritonitis were found, and yet from the peritoneal exudate and blood the bacteriophage was recovered. All of these experiments have been repeated several times and our results have been consistent. The results seem to offer encouragement for further experiments in this field.

6061

Return of Gastric Acidity after Subtotal Gastrectomy and Double Vagotomy.

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The following study was undertaken to determine the influence of subtotal gastric resection and double vagotomy upon gastric acidity in dogs. Portis and Portis[1] showed that the total acidity remained unaltered after subtotal gastrectomy in dogs. Hartzell[2] found a marked reduction in acid after supra-phrenic double vagotomy, but 2 years later Vanzant[3] working with the same animals reported that the acid had returned to normal values.

Complete studies have been made upon 5 dogs. Preliminary to resection and vagotomy, a Pavlov pouch was made. Then, 2 to 3 weeks later when the acidity of the pouch was stabilized, the second operation was performed. This consisted of isolation and division of the anterior and posterior vagal trunks on the abdominal portion of the esophagus and resection of the distal portion of the stomach from a point approximately 3 cm. proximal to the *incisura angu-*

[1] Portis, S. A., and Portis, B., *J. Am. Med. Assn.*, 1926, **86**, 836.
[2] Hartzell, J. B., *Am. J. Physiol.*, 1929, **91**, 161.
[3] Vanzant, F. R., *Am. J. Physiol.*, 1932, **99**, 375.

laris to just beyond the pyloric ring, thus insuring complete removal of the antrum and pylorus. The stump of the duodenum was closed and an anterior Polya gastro-jejunostomy was established. For the study of gastric secretion a test meal consisting of lean meat and water was used. Specimens were obtained for analysis from the intact stomach before operation; from the pouch and main stomach 12 days after the first operation; and from the pouch and gastric remnant for 6 to 12 weeks after the second operation. The samples were aspirated at hourly intervals for periods of 4 to 7 hours after the administration of the test meal and were examined for free, combined, and total acidity by titrating against N/10 sodium hydroxide. In addition, the effect of atropine upon the secretory response to a test meal was studied before and after subtotal removal of the stomach and section of the vagi.

The following results were obtained. Before gastric resection and vagotomy, maximum total acidities of 85 to 100 clinical units were reached in the main stomach. Higher figures up to 100 and 120 units were present in the secretion from the pouch. Two mg. of atropine reduced the acidity about 50%, but after a few hours the acid returned to its original level. The highly acid juice which poured out of the pouch began to digest the adjacent skin and only daily neutralizing dressings controlled this action.

After gastrectomy and vagotomy the acidity was diminished for a period of 2 to 4 weeks. The maximum total acidity in the main stomach remnant in response to the test meal dropped to 30 or 40 clinical units and in the pouch to 40 or 50 units. On account of the widely open Polya stoma, biliary and duodenal regurgitation were at times so marked as to depress gastric acidity even further. At this period, so little acid was discharged by the pouch that the dressings could be discarded and whatever digestion of the skin had been present before the operation quickly healed. After this interval however, gastric acidity began to increase steadily in all 5 dogs. The skin about the pouch began to break down again and careful daily neutralizing dressings had to be resumed. Within 4 to 6 weeks after the operation, maximum total acidities of 85 to 100 units were again obtained from the main stomach remnant, and 120 to 130 units from the pouch. The only final difference in the secretory curves obtained before and after resection and vagotomy was that in 3 dogs the highest acidities appeared 3 to 4 hours after the test meal was given, instead of after one to 2 hours. The injection of atropine caused about the same degree of temporary inhibition of gastric secretion as before gastrectomy and nerve di-

vision. Up to the present, 6 to 12 weeks after the resection-vagotomy operations, the return of hydrochloric acid to preoperative values has persisted in all of the animals except when an occasional biliary regurgitation modifies it (observed in 1 dog). In the main stomach or its remnant the amount of free hydrochloric acid in the contents after a test meal varied with the degree of neutralization by regurgitated intestinal fluids, and the amount of fixation of the acid radical by the protein in the meal and the protein in the mucus secreted by the gastric glands. On the other hand, in the pouch the secretion elicited after a test meal consisted nearly entirely of free hydrochloric acid, only a small amount being bound by the protein in the mucus.

The results of these experiments show that in the dog, subtotal gastrectomy combined with double infra-phrenic vagotomy induces only a temporary reduction in gastric acidity which is followed by a complete return of secretory function. Apparently the stomach is endowed with highly efficient compensatory mechanisms which provide for the continued production of hydrochloric acid under such conditions. The fact that the division of both vagi does not abolish the action of atropine indicates that the intrinsic postganglionic vagus mechanism remains intact.

6062

Further Studies on Continuous Secretion of the Pancreas.

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In a previous communication[1] observations were presented which indicate that in the dog pancreatic secretion is continuous during interdigestive periods, as it is in rabbits and ruminants. In another paper[2] we have discussed the inhibitory effect of ether anesthesia on continuous secretion, which explains the absence of continuous flow in the acute experiments of Bayliss and Starling. It has been claimed that continuous secretion in permanent fistulas is

[1] Berg, B. N., and Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 724.

[2] Zucker, T. F., Newburger, P. G., and Berg, B. N., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 294.

due to lack of neutralization of gastric hydrochloric acid by the pancreatic juice diverted from the intestine.[3]

In order to determine whether this factor has an effect on continuous secretion the following experiment was performed. A dog was prepared with a system of altercursive intubation as described by Elman and McCaughan[3] by means of which the pancreatic juice was continuously returned to the intestine through the biliary tract. This consists of an intercommunicating system of tubes leading from the pancreas to the gall bladder in such a way that the flow of juice can be observed on the outside. By this procedure the pancreatic secretion was allowed to enter the duodenum through the ampulla of Vater and neutralization of the gastric juice could take place as in the normal animal. Elman[4] states that the gastric hypersecretion which he reports as accompanying complete drainage of pancreatic juice to the outside, does not occur under these conditions.

Two types of experiments were performed on dogs with altercursive intubation. A T-tube manometer was inserted in the outer circuit to measure the maximum pressure developed during secretion and a dropping bulb to count the number of drops secreted per minute was arranged in such a way that the juice from the pancreas appeared in drops from a jet at the top of the bulb and was returned from the bottom of the bulb to the intestine via the gall bladder. Our observations show clearly that with uninterrupted return of juice to the intestine, continuous secretion could be demonstrated even in animals deprived of food for more than 24 hours. The pressure measurements were of the same order of magnitude as with complete drainage to the outside previously recorded. The fasting secretion developed a pressure equivalent to about 280 mm. of juice and after giving food this rose to about 320 mm.

Therefore, unless we want to assume that all procedures, such as intubation of ducts, lead to grossly erroneous results, we must conclude that with experimental conditions as nearly normal as possible, the pancreatic secretion of the dog is continuous during fasting. At any rate, all explanations of continuous secretion as an abnormal effect due to lack of neutralization of gastric hydrochloric acid become untenable.

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[3] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

[4] Elman, R., *Arch. Surg.*, 1928, **16**, 1256.

6063

Precipitin Reactions of Immune Sera with Simple Chemical Substances.

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It has been shown[1] that specific serological reactions can be obtained with simple chemical substances, consisting of an inhibitory effect upon the precipitation of azoproteins containing corresponding chemical groups. The explanation of these inhibition reactions was obviously that the simple substances combine with the antibodies, thereby preventing the formation of a precipitate with the antigen. Direct evidence of such a combination has been claimed by Klopstock and Selter.[2] Recently, Marrack and Smith[3] have shown that on addition of the corresponding immune serum, less of an azodye made from p-aminobenzene arsinic acid passed through a collodion membrane than when a normal serum was added. The specificity of the phenomenon was checked with another azodye (methyl red).

We have found that it is possible to obtain direct precipitation reactions with substances of simple chemical constitution.* The immune sera used were prepared by injecting azoproteins made from the para-amino derivatives of succinanilic, adipanilic, and suberanilic acids. For the reactions, azodyes were used made by diazotizing the compounds mentioned above, and coupling with resorcinol or tyrosine. The concentration of the test solutions was 1:10,000 and 1:50,000.

In their specificity the reactions, particularly the group reactions of the dyes made from p-amino-adipanilic and p-amino-suberanilic acid, correspond fully to the reactions observed with azoproteins, which will be described in a later communication. There was a further correspondence in that in both cases the reactions could be

[1] Landsteiner, K., *Biochem. Z.*, 1920, **104**, 280; Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1928, **48**, 315; 1929, **50**, 407; 1931, **54**, 295; Klopstock, A., and Selter, G. E., *Z. Immunitätsf.*, 1928, **55**, 118; Avery, O., and Goebel, W. F., *J. Exp. Med.*, 1929, **50**, 521.

[2] Klopstock, A., and Selter, W. F., *Z. Immunitätsf.*, 1928, **57**, 174.

[3] Marrack, J. R., and Smith, F. C., *Nature*, Dec. 26, 1931, p. 1077.

* In this connection mention may be made also of experiments in which the injection of azodyes caused anaphylactic shock, although not regularly, in guinea pigs sensitized with azoproteins (PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 811, and *J. Exp. Med.*, 1930, **52**, 347.

TABLE

To 0.2 cc. of a solution of the sodium salts of the dyes (prepared with resorcinol) in saline in a concentration of 0.01 or 0.002%, 1 or 2 capillary drops of immune serum were added. Readings were taken after 2 hours at room temperature and after standing over night in the icebox. The intensity of the reactions is indicated as follows: 0, ftr (faint trace), tr (trace), *tr* (strong trace), ±, +, +±, etc.

Immune sera for azoproteins made from	Reading taken after	Dyes made from: p-amino succinanilic acid 0.01%	p-amino succinanilic acid 0.002%	p-amino adipanilic acid 0.01%	p-amino adipanilic acid 0.002%	p-amino suberanilic acid 0.01%	p-amino suberanilic acid 0.002%
p-amino-succinanilic acid 1 drop	2 hours	tr	+	0	0	0	0
	Night in icebox	+	++	0	0	0	0
p-amino-adipanilic acid 2 drops	2 hours	0	0	*tr*	*tr*	0	0
	Night in icebox	0	0	+±	+	tr	0
p-amino-suberanilic acid 1 drop	2 hours	0	0	±	±	++	++
	Night in icebox	0	0	+±	+±	+++	++±

inhibited specifically by the addition of the nitroanilic acids. Weak but definite precipitin reactions were also obtained with azodyes prepared from aminotartranilic acid and p-arsanilic acid, and the homologous immune sera.

6064

Blood Losses in Experimental Intestinal Strangulations and Their Relationship to Degree of Shock and Death.*

HORACE G. SCOTT AND OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

Most investigators and clinicians believe that death in intestinal strangulation is due to toxemia resulting from absorption of toxic products from the lumen or wall of the strangulated bowel.[1, 2] It was previously observed that the fall in blood pressure was intimately correlated with the type, length (of bowel) and duration of strangulation.[3] In this study the blood losses accompanying varying types of strangulation will be detailed.

* This work was supported in part by Grant 244 allowed by the Committee on Scientific Research of the American Medical Association.

1 VonAlbeck, *Arch. f. klin. Chir.*, 1902, **65**, 569.

2 Murphy, Fred T., and Vincent, Beth, *Boston Med. and Surg. J.*, 1911, **165**, 684.

Methods. The data presented here on blood volume losses were obtained from experiments on 38 animals. As in the other phases of this work[3, 4] the intestinal strangulations were divided into 4 groups. *Group I.* 11 dogs. The veins to varying lengths of intestine (3-5½ ft.) were completely tied off and the animals were autopsied immediately after death. *Group II.* 11 animals. An encircling ligature was passed about the bowel wall and mesenteric pedicle to loops of bowel of varying length (1-5 ft.), and the loops resected when the animals appeared moribund 2 to 5 hours later. In this group the degree of venous and arterial occlusion depended entirely upon the tightness of the constricting mechanisms. In practically every case the venous occlusion was complete while the arterial occlusion was only partial. *Group III.* 9 animals. The arteries and veins to loops of bowel varying between one and 5 feet were completely ligated and severed and the dogs were autopsied immediately after death. *Group IV.* 7 animals. The arteries to one to 4 foot loops were completely ligated, the veins being left intact, the animals autopsied shortly after death.

At necropsy, in each instance, the peritoneal fluid was measured; the gut with its contents was weighed; the contents were removed and the gut reweighed. The gut length was measured just prior to the strangulation and in some instances afterwards as well. The amount of blood lost was calculated as being equivalent to the peritoneal fluid plus the increase in gut weight over the calculated normal. The reasons for believing that these figures give an estimate of the true blood losses in the first 2 groups can be seen from a careful study of the results. The blood volume loss was calculated from these figures, basing the total blood volume on an arbitrary figure of 7.5% of the body weight, this figure being given by most authors as the maximum figure for total blood volume.

Results. In Group I, venous ligations, shock symptoms and death resulted very soon. Within 2 hours the animals began to show a definite increase in the respiratory and cardiac rates. The hemoglobin had begun to fall as well as the blood pressure. In a few animals in which the abdomen was opened after this time interval the strangulated gut was seen to be of a dark mahogany color, lusterless, and distended. The distension was due to a dark, bloody fluid which had accumulated within the wall and lumen of the bowel.

[3] Scott, H. G., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 748.

[4] Scott, H. G., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 748.

Hemoglobin determinations revealed this fluid to have a hemoglobin content ranging from 60 to 140% (Sahli) of normal blood. The peritoneal cavity usually contained a bloody serous fluid. Hemoglobin content was usually less than 10%, but the total protein content was always about that of the animal's own blood plasma. At the time of death, gross and microscopic sections of the gut revealed the wall to be packed with red blood cells. Weight determinations of the empty gut showed that the wall was so filled with blood that the weight of the gut had increased from 150 to 500% in every instance. All these findings were minimal shortly after producing the strangulation and increased during the course of the strangulation, reaching a maximum at the time of death. Death in Group I occurred after a loss of from 34 to 66% of the total blood volume. The average for the group was 55% and the average length of life was 5½ hours. In Group II, encirclement strangulation, in which there was only partial arterial occlusion and the loops were resected before the animals died the blood volume losses were less, varying from 20 to 50%, with an average of 35% for the entire group. In Groups III and IV in which there was complete arterial occlusion the gut was found to be ruptured in 13 out of 16 instances. In those instances where it was not ruptured there was very little fluid within the lumen. In none of the dogs of these last 2 groups was there found to be any increase in the gut weight of the strangulated loop over the normal. Consequently no figures are listed for these 2 groups with reference to the gut weight.

In those instances where the loops were ruptured, the peritoneal cavity was found to contain rather large amounts of peritoneal fluid of rather high total protein content, the values ranging from that of the animal's own blood plasma up to twice the amount. In the few instances in which the loops were not ruptured the peritoneal fluid had a total protein content less than that of the blood plasma. The amount of fluid found in the peritoneum was calculated to be 22% of the total blood volume in the third group and 20% in the fourth group. A summary of the data will be found in the table.

Comment. The rapid fall in blood pressure, early shock, and death in Group I are associated with a loss of blood sufficient in itself to produce these results. The same is true of Group II. We feel, therefore, that the results obtained in Groups I and II confirm the work of Blalock,[5] who contends that the fall in blood pressure in shock is due to blood losses. However, in Groups III and IV in

[5] Blalock, Alfred, *Arch. Surg.*, 1930, **20**, 959.

TABLE I.

		Dog wt. kilos	Gut length ft.	Strang. time hr.	Peritoneal fluid cc.	Gut wt. gm.	Normal gut wt. gm.	Increase gut wt. %	Total blood loss gm.	Blood volume loss %
Group I	Minimum	8	3.0	2.5	30	260	90	150	260	34
	Maximum	22	5.5	12.0	300	1030	170	490	1015	66
	Average	15	4.3	5.5	96	680	135	283	643	55
Group II	Minimum	11	2.0	2.0	30	240	76	133	180	20
	Maximum	21	5.0	5.5	140	675	150	320	635	50
	Average	15	3.2	4.0	82	460	103	235	425	35
Group III	Minimum	5	1.0	15.0	40	—	—	—	—	48
	Maximum	14	5.0	32.0	325	—	—	—	—	48
	Average	9	2.5	20.0	142	—	—	—	—	22
Group IV	Minimum	8	1.0	15.0	40	—	—	—	—	14
	Maximum	20	4.0	24.0	350	—	—	—	—	30
	Average	15	2.5	20.0	250	—	—	—	—	20

which the arteries are ligated some factors other than whole blood losses must apparently enter in to play the major rôle in the cause of death.

Summary. Data are submitted on 38 animals in which 4 types of strangulation obstruction were produced and followed until death ensued in 3 of the 4 groups. In Group II, the strangulated loops were resected before death, at a time when the animals appeared moribund.

In the first 2 groups in which the veins were occluded, the arteries were patent or only partially occluded. In these groups the animals apparently died from loss of blood into the bowel and peritoneal cavity. In the last 2 groups in which the arterial occlusion was complete the animals lived about 4 times as long, and undoubtedly died from factors other than the loss of whole blood. Just what caused death in these last 2 groups we are unable to say at this time.

6065

A Technic that Facilitates Lumbar Puncture in the Dog.

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In the last year we studied spinal anesthesia for the dog, and a number of difficulties have been overcome by the utilization of a simple piece of apparatus and the adoption of a certain routine in

making the puncture. Our first problem was the development of a method of maintaining the dog in a proper position for the injection. The vertebral interspaces of the dog are very narrow, as compared to those of man and the insertion of the needle is exceedingly difficult unless the dog is kept in a flexed position. In experiments in which premedication with sedatives is inadvisable we have found that the dog can be kept quiet in a comfortable position by the use of a modification of the Delahanty table. This apparatus is readily prepared by the use of a board measuring 1 inch thick, 12 inches wide and 5 feet long. As in the Delahanty table 4 holes are cut through the board in such a manner that the dog's legs may

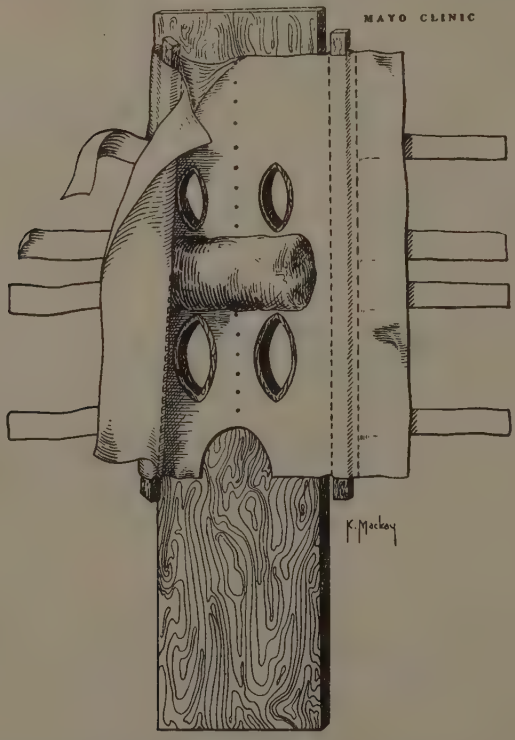

FIG. 1.

A diagram of the essential features of the modified Delahanty table. Four holes are cut through a board 1 foot wide by 5 feet long. The holes are so spaced that the dog's leg can be placed in them. A canvas corset is attached to the board medially. A pillow is placed in front of the hind legs and the corset is drawn about the dog and tied along the dorsum. The pillow maintains the spinal column in a flexed position.

be easily put through them. To insure maximal comfort the holes should be padded. A pillow which maintains the spinal column in a flexed position is placed under the belly. Another pillow is placed between the fore and hind limbs after which the latter are secured. A canvas corset which is attached to the median line of the board is bound about the animal and tied along the dorsum. Lateral movement is reduced by a wooden slat inserted along the corset on each side. By means of this apparatus the dog may be inclined at any desired angle. When the board is placed at an angle of about 45°, flow of spinal fluid is facilitated (Fig. 1). The puncture is preceded by infiltration of the skin and muscles with 0.5% novocain, the injection being carried down to and including the intervertebral space at the level at which the puncture is to be made. A small amount is also injected against and through the dura.

We have found the apparatus and procedure just described of so much assistance that in our hands lumbar puncture in the dog is a relatively easy procedure.

6066

Direct Observations on the Mechanism of Pain in Duodenal Ulcer.

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From the Departments of Surgery and Medicine, University of Chicago.

An extensive literature has developed with respect to the mechanism of pain in gastric and duodenal ulcer. For this reason, in this brief report, we shall make no reference to previous studies but merely describe some observations made during the course of an operation under local anesthesia upon a patient with a duodenal ulcer.

The patient, a well developed white male 37 years of age, first experienced epigastric distress of the ulcer type in 1920. A diet of bland food together with the daily ingestion of powders (presumably alkalies) afforded some relief, but in April, 1922, his distress became so marked that an operation was advised and performed elsewhere. At operation a duodenal ulcer was found and a posterior gastro-enterostomy done. This gave more or less complete relief until 1924, at which time epigastric pain returned more severe than before and persisted intermittently until we first saw the patient in March, 1928. The symptoms and findings at this time were those of a gastro-jejunal ulcer. In April, 1928, the abdomen was opened

by one of us (L.R.D.). A jejunal ulcer was found as well as the scar of an old ulcer on the anterior wall of the duodenum about 1½ cm. distal to the pylorus. There was no evident pyloric stenosis. The jejunal ulcer was excised, the gastro-enterostomy disconnected, and the openings in the stomach and jejunum closed. Following this operation the patient remained free from distress only until October, 1928, when he returned to the hospital, this time complaining of symptoms similar to those of 1920. The periods of distress bore the time relations to food taking characteristic of duodenal ulcer and the distress itself was completely relieved by food and adequate alkalies. On accurate medical management he remained entirely comfortable but after leaving the hospital he was unable to continue treatment and returned in January, 1932. At this time he presented a symptomatology and picture of epigastric distress quite characteristic of ulcer and the introduction of 0.5% HCl by stomach tube was repeatedly found to initiate and reproduce faithfully this same distress. This "acid test" has been found by one of us (W.L.P.) to be positive in a large proportion of such ulcers. It should probably be emphasized that the introduction of 0.5% HCl into the stomach, several hours before the observations described below were made, reproduced the typical pain in severe form, thus indicating that the pain-producing mechanism was very sensitive.

The patient desired surgical relief and, since it was quite apparent that he was not following with sufficient accuracy the medical management prescribed, a partial gastrectomy was advised. The operation was performed January 12, 1932, under local infiltration anesthesia, using ½% novocaine and adrenalin. The abdomen was opened and the stomach exposed with a minimum of discomfort. At this time he felt no ulcer distress. A puckered scar was visible on the anterior wall of the duodenum about 1 cm. distal to the pylorus. On very gently rubbing the serosa over this scar with the gloved finger, the patient complained of pain similar to his ulcer distress. This pain persisted after the rubbing was discontinued. The patient was then told that something would be done to entirely relieve his distress, whereupon the region of the ulcer was rather firmly compressed between the thumb and forefinger of the operator and massaged gently but firmly. This produced severe distress. Several guide threads of fine silk were now introduced into the anterior wall of the pyloric antrum and, by means of these, traction was made on the duodenum, pulling it toward the left. Every time this traction was made, the patient complained of severe pain resembling his ulcer distress.

While this distress was present and while the traction was continued, 20 cc. of 5% sodium bicarbonate solution were injected by means of a hypodermic needle into the lumen of the pylorus. The distress was almost immediately relieved and this relief persisted for about 5 minutes. Twenty cc. of 0.5% HCl were then injected into the first part of the duodenum in the same way and almost immediately the patient complained of a burning type of pain. This persisted until an injection of sodium bicarbonate solution was made. The relief obtained from this last injection was not so striking as at first nor did it persist. After about 3 minutes the patient complained of severe cramping pain which radiated up into his chest. This radiation had been frequently noted before in association with the cramping type of pain. Simultaneous with the appearance of this cramping pain there appeared a deep circular contraction ring just distal to the ulcer. This local spasm of the duodenum after a while passed distally only to be succeeded by several subsequent similar spasms. All during this time the patient complained of very severe cramp-like pain. It is interesting in view of this observation that several peristaltic waves were seen passing over the pyloric antrum at a time when no distress was experienced.

The distress was now so great that the remainder of the operation, a partial gastrectomy of the Polya type was completed under general anesthesia. A chronic ulcer about 2 cm. in diameter was found on the anterior wall of the first part of the duodenum immediately beneath the scar.

These observations show that typical ulcer pain and distress can be produced by mechanical stimulation of the ulcer region in the duodenum by massage or traction. They show in addition that distress originating from such an ulcer may be relieved by the application of alkali to the mucosal surface of the ulcer and that, furthermore, distress can be produced again by the exposure of the ulcer to 0.5% HCl. This distress is not associated with a visible contraction of the stomach or duodenum. A peristaltic wave in the region of the pyloric antrum was observed at a time when the patient felt no ulcer distress. The appearance, however, of severe cramp-like pain simultaneously with the occurrence of marked contraction of the circular musculature of the duodenum in the immediate region of the ulcer certainly suggests that this type of ulcer pain may be produced by muscular spasm at the site of the lesion. It should be noted that following the last injection of the sodium bicarbonate solution into the duodenum the patient complained bitterly of nausea.

6067

Absorption of Insulin by Nasal Mucous Membrane.*

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(With the technical assistance of Milton Landowne and Jac Siegel.)

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The hypodermic injection is the undesirable feature in insulin therapy, and therefore limits its usefulness. Many workers have used oral therapy, viz.: (1) Administering insulin by all enteral and respiratory routes.[1] (2) Adding to insulin: serum, defibrinated blood, bile acids, bile salts, acids, or alcohol, to prevent its destruction or inactivation by the proteolytic ferments of the gastro-intestinal tract.[2] (3) Employing substitutes which possess insulin-like properties.[3] (4) Incorporating the insulin in enteric coated capsules to make it available for absorption after passing the stomach and jejunum.[4] All these have proven to be either toxic or of dubious value.[5]

We approached the problem by altering the permeability of the

* We wish to express our appreciation to Frederick Stearns Co., for their kindness is supplying us with the insulin necessary for this study.

1 Mauriac, P., and Gandy, A., *Compt. rend. Soc. de Biol.*, 1925, **93**, 1524. Harrison, G. A., *Quart. J. Med.*, 1927, **20**, 187. Christie, C. D., and Hanzal, R. F., *J. Clin. Invest.*, 1931, **10**, 787. Peskind, S., *J. Metab. Research*, 1924, **6**, 207. Wassermeyer, H., and Schäfer, A., *Klin. Wchn.*, 1929, **8**, 210. Korbsch, J., *Klin. Wchn.*, 1925, **4**, 2327. Miller, H. R., *Arch. Int. Med.*, 1926, **38**, 779. Bernhardt, H., and Strauch, C. B., *Z. f. Klin. Med.*, 1926, **104**, 767. Lévy, M. M., and Cordier, P., *Compt. rend. Soc. de Biol.*, 1925, **95**, 248.

2 Winters, L. B., *J. Physiol.*, 1923, **58**, 18. Bollmann, J. L., and Mann, F. C., *Am. J. Med. Sci.*, 1932, **183**, 33. Lasch, F., and Brügel, S., *Arch. f. Exp. Path. u. Pharm.*, 1927, **120**, 144. Horsters, H., and Rothmann, H., *Arch. f. Exp. Path. u. Pharm.*, 1929, **142**, 261.

3 Watanabe, C. K., *J. Biol. Chem.*, 1918, **65**, 253. Frank, E., Nothmann, M., and Wagner, A., *Klin. Wchn.*, 1926, **5**, 2100. Von Noorden, C., *Klin. Wchn.*, 1927, **6**, 1041. Blotner, H., and Murphy, W. P., *J. Am. Med. Assn.*, 1927, **94**, 1811. Collip, J. B., *J. Biol. Chem.*, 1923, **56**, 513. Allen, F. M., *J. Am. Med. Assn.*, 1927, **89**, 1577. Stein, H. B., Longwell, B. B., and Lewis, R. C., *Arch. Int. Med.*, 1931, **48**, 313. Leclere, H., *Presse Med.*, 1928, **36**, 1634. Kahnt, K., *Med. Welt.*, 1931, **5**, 886. Nye, J., and Fitzgerald, S., *Med. J. Australia*, 1928, **2**, 626. Gunn, J., and Morrison, D., *So. African Med. J.*, 1924, **22**, 522. Geiger, E., *Fort. d. Therap.*, 1931, **7**, 257. Long, M. L., and Bischoff, F., *J. Pharm. and Exp. Therap.*, 1930, **38**, 313. Cammidge, P. J., *Brit. Med. J.*, 1925, **2**, 1216; *Compt. rendu Soc. de Biol.*, 1930, **104**, 1029. John, H. J., *J. Metab. Research*, 1922, **7**, 489.

4 Murlin, J. R., Sutter, C. C., Allen, R. S., and Piper, H. A., *Endocrinology*, 1924, **8**, 331. Murlin, J. R., and Gaebler, O. H., *J. Biol. Chem.*, 1925, **66**, 731.

5 Guttman, J., and Kallfelz, F., *Klin. Wchn.*, 1929, **8**, 2246.

membrane to stimulate absorption of the insulin molecule. The method was based upon the following principles: 1. The membrane should not be exposed to ferments that inactivate insulin. 2. Permeability may be increased by elevating the temperature of the membrane.[6] 3. The rate of absorption can be speeded by heating the insulin solution. 4. A "piling up" of the molecule on the membrane may be prevented by lowering the surface tension of the insulin solution. 5. All adhering materials should be mechanically washed away from the membrane. 6. The surface of the membrane should be kept at a favorable pH.

The method consisted of: (1) The nose was irrigated with normal saline, at approximately pH 4, at 40-45°C. This was immediately followed by: (2) Either a solution of Saponin was applied to the mucous membrane, or a few drops of Saponin added to the insulin solution. (3) Insulin at 50°C., was then sprayed directly into the nose with an atomizer, the stem of which had been previously heated. (4) Small plegets of cotton were then inserted into the nares to prevent the patient from blowing out the solution.

100 units of U 100 insulin were employed in all experiments. The subjects were human diabetics. The experiments were performed in the post-absorptive period, at least 14 hours after the last meal. The blood sugars were determined during control periods of 1-3 hours before the application. Blood sugars were usually determined at 5, 15, 30, 60, 120, 180, and 240 minutes after the application, by the Folin-Wu method, in duplicate. No results were acceptable where the deviation between the duplicates was greater than 2%. Urinary sugars were estimated by the Benedict quantitative method.

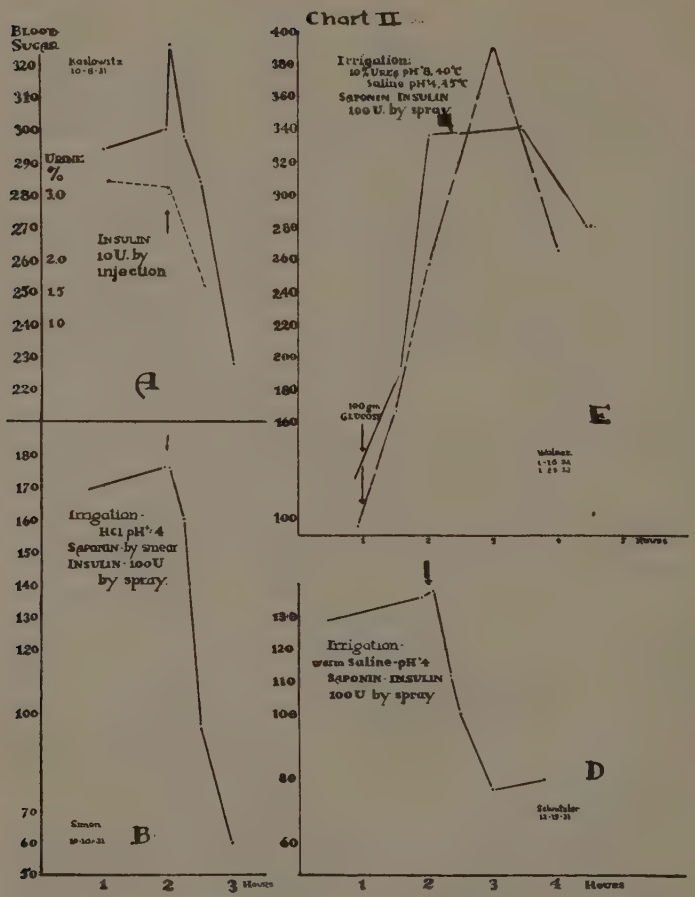
Illustrative examples of the results are given in Chart II. Ninety-six experiments were performed, with 11 failures, due either to poor irrigations, cooling of solutions, or incomplete spraying.

Mosenthal[7] reported that diabetics, if starved long enough, showed a progressive decline in blood sugar to a normal level. Macleod[8] has been confirmed in his observation that maximum depression in the blood sugar occurs within 60 minutes in the normal, and within 2 hours in the diabetic, regardless of insulin dose, or whether administered subcutaneously or intravenously. Therefore, to be sure of an insulin effect, one must know the preliminary curve

[6] Osterhout, W. J. V., Injury, Recovery and Death, in Relation to Conductivity and Permeability, Lippincott, 1922.

[7] Mosenthal, H. O., Tice-Practice of Medicine, Hagerstown, 1928, 69.

[8] Macleod, J. J. R., Carb. Metab. and Insulin, Longmans Green, 1926, 270.



A—Blood and urine sugars after the hypodermic injection of 10 units of insulin. B—Shows parallel precipitous drop in blood sugar after nasal treatment. E—After 100 gm. of glucose by mouth (broken line). One week later, same patient, 100 gm. of glucose, followed by nasal treatment. Note sudden interruption in the rise of the curve (full line). D—Is a characteristic curve produced by this method of therapy.

of the blood sugar, obtain a precipitous depression, and a maximum depression within 2 hours. Any attempt to interprete blood sugar depressions over longer periods of time subjects one to the possible criticism that it might be a starvation effect. Therefore the results of so many experiments in this field can not be adequately interpreted. Our method of nasal treatment produces curves which fulfill

every critical requirement, and strongly resemble those obtained by the subcutaneous injection of insulin.

Its clinical application is limited because this treatment produces a mild congestion in the mucous membrane of the nose, and symptoms of rhinitis. These symptoms usually last about one hour. Before this method is available for clinical use, one must establish the proper dosage. The authors are now investigating these problems.

6068

Antiurease Formation in the Hen.

STACEY F. HOWELL. (Introduced by James B. Sumner.)

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Although urease is very toxic when injected into mammals it has very little effect upon the hen unless urea is injected simultaneously. This is to be accounted for by the low urea content of hen's blood, which has been found to be about 1 mg. per 100 cc., or even less, in the 8 hens tested. From 800 to 17,000 units of urease were injected at one time, into a wing vein or directly into the heart. The white leghorn hens were observed following the injection and samples of blood and feces were analyzed for ammonia, urea, uric acid and urease. The injected urease disappeared from the blood within 4 hours after the injection and did not appear in the feces. Following injection of urease the blood urea disappeared entirely, its place being taken by ammonia. The uric acid content of hens' blood (2.0-4.0 mg. per 100 cc.) was not apparently affected by destruction of the urea, as might be expected if urea were a necessary precursor of uric acid. Examination of the feces showed that the reaction becomes slightly alkaline after the injection of urease.

Antiurease was formed in 8 hens by 4 to 10 injections of urease (each injection containing from 500 to 5,000 units) over a period of 30 to 50 days. Antiurease could be demonstrated in the blood about 14 days after the first injection. There was an incubation period of 7 days after the last injection. The amount of antiurease found in the hens, as determined by the method of Kirk and Sumner,[1] varied between 5 and 24 antiunits per cc. of serum. The chicken

[1] Kirk, J. S., and Sumner, J. B., *J. Biol. Chem.*, 1931, **94**, 21.

antiurease which has been obtained is similar to rabbit antiurease. It protects rabbits from a fatal dose of urease, inhibits the action of urease on urea and can be recovered and purified by the method described by Sumner and Kirk.[2]

One of the most important points brought out by this work is that although urease does not cause visible poisoning in the hen, nevertheless antiurease is formed.

6069

Occurrence of a Silico-carbohydrate Derivative in Animal Tissue.

J. M. JOHLIN.

From the Department of Biochemistry, Vanderbilt University School of Medicine, Nashville.

Although silicic acid has long been known to occur in practically every tissue of the living organism there has been no evidence that it is in combination with organic matter as are, for example, sulphuric and phosphoric acids. Drechsel[1] long ago reported its occurrence as an ester of cholesterol in bird feathers but there has been no confirmation of his observation since.

Kraut[2] has recently reported that the silicic acid content of human blood is constant in any one individual at different times, that it varies considerably in different individuals and that a temporary variation can be brought about in any one individual by the administration of silicates.

By the electro-dialysis of gelatin, ox tendon, horse and ox-blood, and human urine, the writer has recovered, at the cathode, small amounts of a jelly-like substance, containing both silicic acid and organic matter, and giving a test for carbohydrates. This substance is insoluble* in water, in 5% hydrochloric acid and in dilute alkali. It chars when heated on platinum and discolors concentrated sulphuric acid when heated. A suspension in water slowly gives the Molisch test for carbohydrates. It is changed by heating with 10%

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[2] Sumner, J. B., and Kirk, J. S., *Zeit. für Physiol. Chem.* in press.

[1] Drechsel, E., *Centrbl. Physiol.*, 1897, **11**, 361.

[2] Kraut, H., *Hoppe Seyler's Z. Physiol. Chem.*, 1931, **194**, 81.

* The substance carried by the electric current is soluble. It appears to become insoluble in the process of its recovery by evaporation following saturation with CO_2.

sodium hydroxide so that the organic matter and a part of the silicic acid go into solution, while the residue now left is soluble in 5% hydrochloric acid and contains calcium, iron and silicic acid. It does not give a test for cholesterol.

The organic fraction of the substance appears to be comparatively small and it cannot be stated to what extent a more complex substance was disintegrated by the electro-dialysis.

Unlike the present substance, colloidal silica is negatively charged and migrates to the anode. The possibility of a gravity drift was guarded against in the present experiments. No distinct particles of the substance recovered in the present experiments could be seen when magnified 1000 diameters, and it can be recovered by electro-dialysis through a collodion membrane which does not permit protein to pass. These facts and the general behavior of this substance make it seem most unlikely that it consists of organic matter adsorbed on colloidal silica.

While working with fairly large amounts of raw material, the amounts of substance recovered, especially from blood and urine, have been very small and larger amounts will be required to determine the more exact nature of this substance and its biological significance.

6070

The Intestinal Flora of Rachitic Rats Before and After Treatment with Ultra-violet Rays.

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Zucker and Matzner[1] showed that when rats develop rickets as the result of being placed on a standard rickets-inducing ration, high in calcium and low in phosphorus, the feces become more alkaline and that on the administration of cod liver oil the reaction veers quickly to the acid side. These observations have been amply substantiated. Grayzel and Miller[2] showed later that the reaction of the intestinal contents of dogs is acid throughout almost the entire

[1] Zucker, T. F., and Matzner, M. J., PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 186.

[2] Grayzel, D. M., and Miller, E. G., *J. Biol. Chem.*, 1928, **76**, 423.

length of the gut, but that this reaction tends to become alkaline when rickets is brought about. As in the case of the rat the reaction reverts to normal on the administration of cod liver oil or treatment with ultra-violet irradiation.

Our investigation was carried out on white rats weighing approximately 50 gm., *i. e.*, about 4 weeks of age. Its main purpose was to ascertain whether rickets leads to a change in the flora of the intestinal tract and whether, after the animal is treated with ultra-violet rays and healing of the rachitic lesion is brought about, there was any associated modification of the flora. The rats were fed the rickets-inducing ration of McCollum and some were irradiated with a mercury vapor lamp. Immediately after the animals were killed by trauma they were autopsied and segments of the intestinal tract were tied off; that of the small intestine extending from the duodenum to the lower ileum and that of the large intestine extending from somewhat below the caecum to the beginning of the rectum. The content of each of these segments was squeezed out and the H-ion reaction obtained as soon as possible by a colorimetric method (Clark and Lub indicators). The material was then suspended to a standard density in normal saline solution, gram-stained films prepared and cultures made by methods adequate to determine quantitatively and qualitatively the character of the flora. A technic described by one of us[3] was followed in part. Whether a flora was acid- or alkali-producing was decided on the basis of the known tendencies of predominant types isolated and also of the reaction, after a 3 or 4 days' incubation, of the surface-seeded lactose bromcresol purple agar plates.

The accompanying table gives the data in regard to 3 bacteriological tests of this kind. The first pair of rats (17529 and 17527) were given a "normal diet", one which was complete in every respect. This diet contains a high percentage of carbohydrate. The whole yellow corn (57%) and the whole dried milk (25%) contain the particular substances, lactose and dextrin, which shift the intestinal flora to a purely fermentative type, more or less dominated by *L. acidophilus*. In surveying the test of these 2 normal animals, one of which was untreated and the other subjected to ultra-violet radiation, it will be noted that there was no distinctive difference between the 2 animals either in the acidity or in the type of intestinal flora, except that *B. coli* was more numerous both in the small and large intestine of the irradiated animal than in the untreated. This

[3] Torrey, J. C., *J. Infect. Dis.*, 1926, **39**, 351.

TABLE I.

Intestinal Flora of Normal, Rachitic and Irradiated Rats.

Rat No.	Diet	Antirachitic irradiation	Radiographs	Source of material cultured	pH	Flora
17529	Normal	None	Normal	Small intestine	6.8	Gm. + predom. *L. acidophilus* ++* *B. coli* + Yeasts + Proteolysis — Acid-producing
				Large intestine	6.9	Gm. — sl. predom. *L. acidoph.* ++++ Yeasts ++ *B. coli* + *Staph. albus* + Proteolysis — Acid-producing
17527	”	Irradiated	”	Small intestine	6.6	Gm. + predom. *L. acidophilus* ++ *B. coli* + Yeasts + g + cocci + sl. Proteolysis — Acid-producing
				Large intestine	7.1	Gm. — sl. predom. *L. acidoph.* +++ *B. coli* ++ Enterococci ++ Proteolysis — Acid-producing
17210	Rickets-inducing	None	Marked rickets	Small intestine	7.2	Gm. + predom. Streptococcus +++ *Staph. albus* ++ *B. coli* + Subtiloid types + *L. acidophilus* + Proteolysis ++ Alkali-producing
				Large intestine	7.2	Gm. + and — about equal Types similar to small intestine *B. coli* ++ *B. proteus* + Proteolysis + Alkali-producing
17213	”	Irradiated	Marked healing	Small intestine	6.5	Gm. + and — about equal *L. acidoph.* +++ *B. coli* ++ *B. proteus* ++ Proteolysis ++ Alkali-producing (?)

* Number of + signs indicates approximately the relative numbers of bacterial types, as shown by culture, for *each* specimen.

TABLE I (Continued)

Rat No.	Diet	Antirachitic irradiation	Radiographs	Source of material cultured	pH	Flora
17213	Rickets inducing	Irradiated	Marked healing	Large intestine	6.8	Gm. — predom. (strong) *B. coli* ++++ Streptococcus + *L. acidophilus* + Proteolysis + Acid-producing
17679	Rickets inducing	None	Marked rickets	Small intestine	6.9	Gm. + predom. Nearly sterile Streptococcus + Yeasts + *L. acidophilus* + Proteolysis —
				Large intestine	7.7	Gm. — predom. Enteroc. and Streptoc. ++++ Coliform alk. +++ Coliform acid + Yeasts + Proteolysis + Alkali-producing
17732	"	Irradiated	Marked healing	Small intestine	6.8	Gm. + predom. Streptoc. and enteroc. +++ *L. acidophilus* + Proteolysis + Sl. acid-producing
				Large intestine	7.0	Gm. — predom. *B. coli,* acid, ++++ Streptococcus + *L. acidophilus* + Proteolysis + Acid-producing

point is worthy of mention because in each pair of rats the same phenomenon was noted, particularly as regards the large intestine.

In the rachitic rats (Nos. 17210, 17213, 17679, 17732) we find a marked distinction between those which had advanced rickets and those which showed marked healing of the epiphyses as the result of ultra-violet rays. First, there is the difference in the reaction of the content of the small and large intestine, particularly of the latter. The McCollum diet stimulated the development of a more complex type of flora than did the normal (Bill's) diet. Not only was there a greater variety of bacteria in both sections of the intestines but in the untreated rachitic rats the alkali-producing tendency of the flora was marked. This was particularly true for the large

intestine. The bacterial types involved seemed to be principally alkali-producing coliform bacilli, *B. proteus* and subtiloid types. Irradiation apparently tended to suppress these types and to encourage the overgrowth of acid-producing *B. coli.* The proteolysis referred to in the table was that caused by spore-bearing anerobes. It will be noted that irradiation apparently had no effect on these organisms.

Irradiation seemed to increase the number of viable bacteria both in the small and large intestine. The exposures in the several tests were of varying intensities, the object being to give a sufficient intensity to bring about marked healing.

A test carried out along the same lines, but one in which calcification at the epiphyses was induced to a lesser degree, furnished information as to whether the change in reaction or in the flora was primary. This experiment may be summarized by the statement that a definite change to the acid side was brought about in the intestinal contents at both levels. In the untreated animal the small intestine showed a pH of 7.2 and the large intestine a pH of 7.7, whereas in the irradiated animal the corresponding values were 6.8 and 6.9. However, in spite of the fact that the contents had been rendered acid as the result of irradiation, there was but slight distinction in the nature of the flora. Enterococci and streptococci predominated strongly within the intestinal canal of the treated as well as of the untreated animal. The sole difference was that the former had rather more *B. coli* in the large intestine. From this experiment and the fact that the flora of the small intestine would seem to be too scanty to play a rôle, we infer that the change in reaction towards acidity, which follows ultra-violet irradiation, is the primary phenomenon and that it is due to an alteration in metabolism and is not the product of bacterial activity. In other words, the acid reaction led to a change of the flora and the flora did not seem to bring about the acid reaction, but may have rendered it more pronounced.

The question arises as to whether similar bacterial alterations occur in connection with the development of infantile rickets and osteomalacia and what effect such changes might have on metabolism, on nutrition and on the intensity of the rachitic process. In infants there is a tendency toward alkalinity of the feces during rickets and likewise a tendency for the reaction to become more acid in the course of the cure of the disorder.

This study will be extended to include other animals and experiments will also be carried out to ascertain the effect on the intestinal

flora of other antirachitic agents, such as cod liver oil and viosterol. It seemed best to begin with a test of the effect of ultra-violet irradiation, as this measure did not necessitate the introduction of any curative substance into the intestinal canal.

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Hemoglobin Regeneration in the Anemic Albino Rat with Dietary Supplements of Spinach, Apricot and Liver.

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Waddell, Elvehjem, Steenbock and Hart[1] showed that anemic rats on a milk diet receiving a supplement of 0.5 mg. iron daily failed to materially increase their hemoglobin levels. Acid extracts of ash residue from liver, lettuce and corn when fed in amounts sufficient to supply 0.5 mg. iron daily, however, induced hemoglobin regeneration. Subsequently, Hart, Steenbock, Waddell and Elvehjem[2] presented evidence that the response elicited by vegetable and meat tissue ash is due to the presence of copper in small but sufficient amounts to act as a supplement to the iron. They also stated that their experiments failed to demonstrate the existence of an organic factor necessary for hemoglobin synthesis. Later work by Elvehjem, Steenbock and Hart[3] failed to substantiate the claim of Drabkin and Miller[4] that glutamic acid may serve to stimulate hemoglobin regeneration in anemic rats on a diet of whole milk with additional iron.

Myers and Beard[5, 6] report hemoglobin regeneration with but 0.25 mg. iron unsupplemented by other metals when fed with a milk diet. Larger doses, and supplemental metals as copper, manganese, arsenic, etc., gave more rapid hemoglobin regeneration.

This paper reports results obtained in experiments on rats with

[1] Waddell, J., Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1928, **77**, 777.

[2] Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

[3] Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197.

[4] Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531.

[5] Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

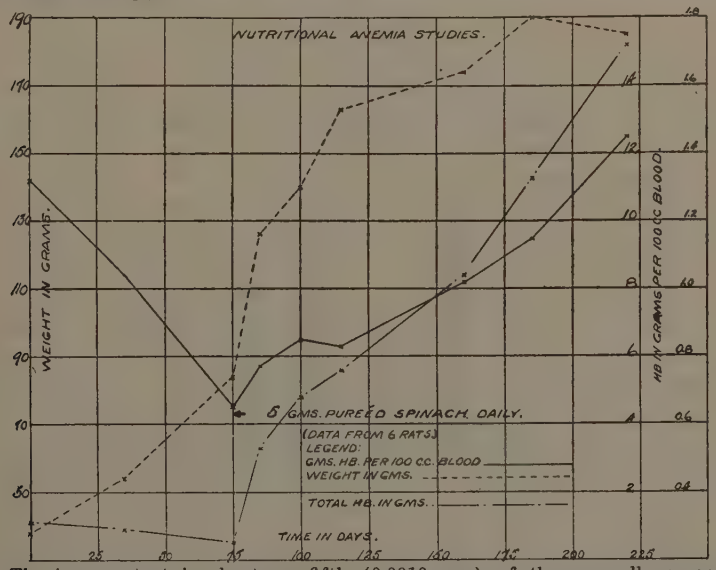
[6] Myers, V. C., and Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 89.

nutritional anemia when a milk regimen is supplemented with spinach, apricot, or liver.

Albino rats at the time of weaning (28 to 30 days old) were placed in individual glass cages[7] on a diet of whole raw cow's milk (collected in glass) for a period of 70 to 80 days. During this interval the hemoglobin content decreased from an average value of 11 to 12 gm. per 100 cc. blood to 3.6 to 4.8 gm. At this time a definite quantity of the supplement was fed daily in a small glass dish, the completeness of consumption being noted. Hemoglobin regeneration was obtained with 5 gm. of pureed spinach daily as shown in Graph I.

GRAPH I.

Hemoglobin Regeneration on Daily Supplement of 5 gm. Pureed Spinach. (Typical Hemoglobin Depletion is Shown During 75 Day Period Prior to Spinach Feeding.)



The iron content is about one-fifth (0.0910 mg.) of the generally accepted optimum. Larger amounts of this supplement (10 and 20 gm. daily) gave more rapid regeneration. The maximum for 20 gm. is, however, but 0.364 mg. iron. The copper content is in all cases equal to or above the accepted minimum.

In experiments conducted with acid solutions of the spinach ash, obtained from 5 gm. of pureed spinach, hemoglobin regeneration was insignificant. A slow response to spinach ash as compared with an equivalent portion of concentrated aqueous spinach extract has been reported by Mitchell and Miller.[8]

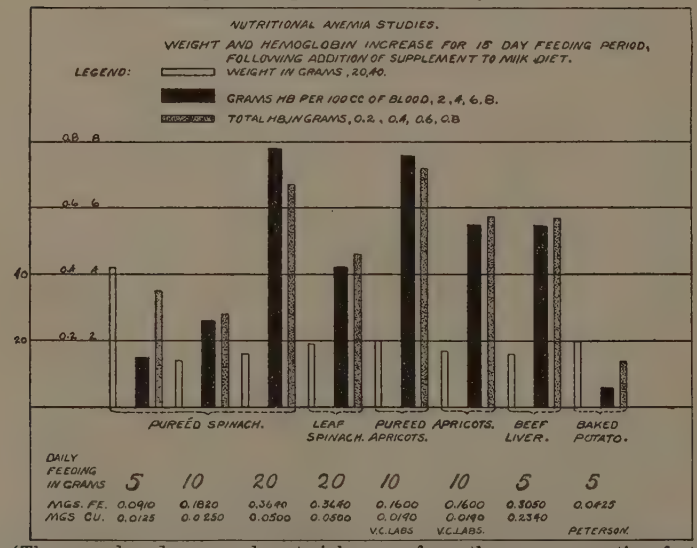
[7] Nevins, W. B., and Shaw, D. D., *Science*, 1930, **72**, 250.

[8] Mitchell, H. S., and Miller, L., *J. Biol. Chem.*, 1929, **85**, 355.

It has been reported by Zih[9] that anemic rabbits will regenerate hemoglobin on optimum doses of chlorophyll, while an overdose causes hemoglobin destruction. In order to check up on the hematopoeitic function of the chlorophyll and mineral factors, extracted chlorophyll from 5 gm. of spinach was fed daily. We were unable to get a favorable response. Our results rather indicate a toxic action with chlorophyll at this level, when fed alone or with the ash equivalent of 5 gm. of pureed spinach.

Robscheit-Robbins and Whipple[10] have recently reported experiments on the relative hematopoietic efficiency of liver, apricot, and spinach in dogs rendered anemic by bleeding. From their results, these substances can be graded in a 4-2-1 ratio. We have found a close approximation to their ratio with the anemic rat as shown in Graph II.

GRAPH II.
Hemoglobin Regeneration in 15 Day Period.



(The pureed and unpureed materials were from the same respective factory batches.)

It should be observed that supplements of pureed spinach or apricot give a greater hemoglobin response during a 15-day period than equal quantities of the non-pureed materials. Since the min-

[9] Zih, A., *Arch. ges. Physiol.*, 1930, **223**, 728.

[10] Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Physiol.*, 1930, **92**, 400.

eral components of these respective materials are the same, our experiments would suggest a better utilization of the finely divided (pureed) material by the animal.

We wish to acknowledge the kindness of Dr. G. A. Fisher of the Van Camp Packing Company in supplying the spinach and apricots for this investigation. All material was sealed in lacquered tin cans. No evidence of corrosion could be detected nor was it indicated by the following analysis:

June 23, 1931, Canned Pureed Spinach	Fe = 0.001820%
Feb. 20, 1932, Canned Leaf Spinach	Fe = 0.001852%
Feb. 20, 1932, Above Spinach Hand Pureed	Fe = 0.001829%
Oct. 4, 1931, Canned Pureed Spinach	Cu = 0.000250%
Feb. 25, 1932, Canned Leaf Spinach	Cu = 0.000236%
Feb. 25, 1932, Above Spinach Hand Pureed	Cu = 0.000246%

All material was from the same original factory batch.

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Nutritional Value of Proteins as Influenced by Exposure to Ultraviolet Irradiation.*

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The experiments described below include (A) *in vitro* digestion experiments on irradiated and non-irradiated proteins with pepsin and trypsin respectively and (B) growth and metabolism studies on rats kept on low protein diets where the protein was either irradiated or non-irradiated.

A. *In vitro* Digestion Experiments on Irradiated and Non-irradiated Purified Proteins with Pepsin and Trypsin, Respectively.

1. Tryptic Digestion of Casein. The irradiated product was prepared by exposing dry casein (Merck according to Hammarsten) for 2½ hours at a distance of 18 inches to the mercury quartz lamp in a current of air. The digestion experiments with irradiated and non-irradiated casein were carried out as described by Helmer.[1] At the end of the digestion period the undigested protein was precipitated and the refractive indices of the filtrates read.

* These researches were supported in part by the Quaker Oats Company.

[1] Helmer, Oscar, Doctor of Philosophy Dissertation, September, 1927, 10, University of Chicago.

As shown in Table I, the non-irradiated casein is somewhat better digested than the irradiated. The refractive index readings were confirmed by actual determination of nitrogen in the filtrates.

TABLE I.

Tryptic Digestion of Irradiated and Non-irradiated Casein as Measured by Changes in Refractive Index and Soluble Nitrogen on Isoelectric Acetic Acid Filtrates.

Mgm. U.S.P. Pancreatin	Refractive Index Readings				Changes in Refractive Index				Mgm. N in 10 cc. filtrate			
	Irrad. 2½ hr.		Non-irrad.		Observed		After cor. for blank		Irradiated		Non-irrad.	
	0 hr.	4 hr.	0.hr.	4 hr.	Irrad.	Non	Irrad.	Non		Cor.		Cor.
					Experiment IV							
0	16.49	16.55	16.52	16.8	0.06	0.28			0.31		0.41	
4	16.5	18.57	16.52	19.0	2.07	2.48	2.01	2.20	1.39	1.08	1.68	1.27
8	16.5	20.6	16.52	21.1	4.1	4.58	4.04	4.3	2.22	1.91	2.74	2.33

2. Peptic Digestion of Egg-White. Dried egg-albumin was prepared and digestion experiments carried out according to the method described by McMeekin and Freeland.[2] A portion of the dried, powdered material was irradiated for 4 hours in a current of air; the longer irradiation period being used because of the coarse physical state of the protein. Here again the amount of digestion was slightly greater for the non-irradiated protein than for the irradiated (Table II).

TABLE II.

Peptic Digestion of Irradiated and Non-irradiated Coagulated Egg White as Measured by Changes in Refractive Index and Soluble Nitrogen on the Acid Solution.

1:3000 U.S.P. Pepsin	Refractive Index Readings				Changes in Refractive Index		Mgm. N in 10 cc. filtrate			
	Irradiated		Non-irrad.							
	2 hr. dig.	4 hr. dig.	2 hr. dig.	4 hr. dig.	Corrected Irrad.	Non-irrad.	Irradiated	Cor.	Non-irrad.	Cor.
0		15.24		15.15			0.3		0.21	
1 mgm.		15.95		15.85	0.71	0.7	3.5	3.2	4.0	3.79
3 mgm.		16.88		16.93	1.64	1.78	8.6	8.3	10.1	9.89
0	15.05		14.87				0.21		0.13	
1 mgm.	15.35		15.48		0.3	0.61	1.65	1.44	1.93	1.8
3 mgm.	15.6		16.1		0.55	1.23	4.32	4.11	5.13	5.0

B. Growth and Metabolism Studies on Rats Kept on Diets Low in Irradiated and Non-irradiated Proteins.

1. Group Experiments. In spite of the very slight differences in digestibility between the irradiated and non-irradiated proteins, the

[2] Freeland, Milnor, Doctor of Philosophy Dissertation, March, 1931, 9. T. L. McMeekin, unpublished data.

final test of actual feeding experiments was obviously the next step. In the usual laboratory diet the protein was reduced to 9% in the hope that at this minimal protein level, slight variations in growth curves could be demonstrated. Four animals, all litter-mates, were given the irradiated casein diet and 4 the non-irradiated. The animals receiving irradiated protein maintained an average weight-curve distinctly superior to those on the non-irradiated casein. Moreover, when the diets were cross-substitued, the growth curves after a short lag, also crossed.

TABLE III.

Tabulation of Results on Metabolism Studies Involving the Feeding of Irradiated and Non-irradiated Casein.

Four rats were studied independently at the same time on each diet. The figures given in this table are the average values. The diets consisted of irradiated or non-irradiated casein 9%, lard 10%, butter fat 15%, salt mixture 4%, dried yeast 5%, corn starch 57%. The casein was irradiated for 2 hours by a Cooper-Hewitt quartz mercury arc lamp at a distance of 40 cm. in a current of air.

Week	Average Gain in weight		Gm. Food Eaten per gm. Gain		Mgm. N Eliminated per gm. Body Wt.		Mgm. N Eliminated per gm. Food Eaten	
	Irrad.	Non-irrad.	Irrad.	Non-irrad.	Irrad.	Non-irrad.	Irrad.	Non-irrad.
First	8.5	7.25	4	5.7	2.07	1.9	3.02	2.52
Second	6.75	4.25	5.4	6.9	3.02	2.64	4.66	4.61
Third	5.5	4.75	5.7	6.0	3.61	3.6	7.21	6.6
Fourth	7	5	4.9	5.6	4.0	3.9	8.07	8.3

2. Individual Metabolism Studies. Finally, to determine whether the improved weight-gain resulting from irradiation of the protein was a result of better nitrogen-retention, metabolism experiments were carried out on individual rats. Four male rats were used for each diet and placed in individual cages. With slight modifications the method of Still and Koch[3] was used for weighing the food and collection of urine and feces. The results are tabulated in Table III.

Average data for the group are given. During the 4 weeks of experiment beginning with the second day after weaning, the gain in weight for the rats receiving irradiated casein was from 0.75 to 2 gm. greater than for those receiving the non-irradiated protein, and at the same time the average food consumption per gm. gain in weight was from 0.3 to 1.7 gm. less on irradiated casein than on non-irradiated. There was no corresponding greater retention of nitrogen, however. The improved weight-gain, therefore, cannot be ascribed to a more economical utilization of the nitrogen.

The limited number of experiments run seems to warrant the fol-

[3] Still, E. U., and Koch, F. C., *Am. J. Physiol.*, 1928, **87**, 225.

lowing tentative conclusions: Irradiation of 2 proteins, casein, and egg-white, in the dry, solid state changes them so that digestion *in vitro* by pepsin and trypsin are slightly inhibited. Metabolism experiments on young male rats, on the other hand, demonstrate a slightly higher body-weight when the protein fraction is irradiated. Since the nitrogen-retention on the low protein diet is slightly less for the irradiated than for the non-irradiated protein, the improvement of the body-weight curve appears to be due to something other than improved utilization of the protein. Vitamin D as an antirachitic agent is ruled out since the diet contains an ample supply for that purpose. Some other factor, stimulating in character is suggested.

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Further Studies on Etiology of Goiter with Particular Reference to the Action of Cyanides*

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The *immediate* cause of thyroid hyperplasia in all probability is a *relative* or an *absolute* deficiency of iodine.[1] The fundamental or essential cause of goiter is unknown, but the search for the essential cause, as we have often suggested, appears to resolve itself into determining the cause or causes of the iodine deficiency. As most iodine deficiencies are relative rather than absolute, the search further limits itself largely to determining the factors which create the increased needs of the organism for the iodine containing hormone. The simplest way of increasing the need of the thyroid for iodine would be by depressing the utilization of oxygen in the tissues, and the discovery by Chesney and Webster[2] that the prolonged feeding of cabbage caused thyroid hyperplasia in rabbits appeared to offer a practical means of testing this hypothesis.

It has been shown that there are great seasonal and climatic variations in the goitrogenic activity of cabbage,[3] that drying in a current

* Aided by a grant from the Ella Sachs Plotz Foundation.

† Fellow of the Rockefeller Foundation.

[1] Marine, D., *Arch. Int. Med.*, 1923, **32**, 811.

[2] Chesney, A. M., Clawson, T. A., and Webster, B., *Bull. Johns Hopkins Hosp.*, 1928, **43**, 261.

[3] Webster, B., Marine, D., and Cipra, A., *J. Exp. Med.*, 1931, **53**, 81.

of air or *in vacuo* causes a loss of the goitrogenic agent,[4] that prolonged steaming does not impair and under certain conditions may increase its goitrogenic power,[5] that boiling for 30 minutes at pH 3.0 (HCl) does not injure it,[6] that the goitrogenic substance may be extracted from cabbage with ether and other ethereal solvents[7] and that this substance is but slightly extracted by prolonged aqueous leaching.

Since all the *Brassicae* so far tested may produce goiter and since mustard oils (isothiocyanates) are the most characteristic constituents of these plants it was thought that their goitrogenic activity might be connected in some way with these substances or with their cyanide precursors. Several of the mustard oils (allyl, ethyl, phenyl) have been fed to rabbits with negative results.

In view of these observations, together with the isolation of nitriles by Hoffmann[8, 9, 10] from several of the *Cruciferae,* the idea that cyanides were the substances which increase the needs of the organism for the thyroid hormone by partially blocking tissue oxidation strongly suggested itself. Accordingly we first tested the least toxic of the cyanides (acetonitrile). When this substance was injected subcutaneously into 4-months-old rabbits daily in doses of 0.1 to 0.25 cc. for 21 days very striking thyroid hyperplasia was produced in animals maintained on a diet of alfalfa hay and oats. In order to determine whether this thyroid reaction was characteristic of cyanides generally we have tested allyl-, propio-, phenylaceto-, phenylpropio- and benzonitrile. Experiments have also been made with phenylisocyanide, potassium cyanide, cyanamide, and sodium thiocyanate. The data of representative experiments are given in Table I.

It will be seen that thyroid hyperplasia may be produced in rabbits by all the cyanides so far tested but in greatly varying degrees. Acetonitrile produced the greatest reaction and the aromatic nitriles as a group the least. These differences were not altogether due to the doses of cyanide. Cyanamide also produced only a slight reaction and sodium thiocyanate none at all.

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[4] Marine, D., Baumann, E. J., Webster, B., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1025.

[5] Marine, D., Baumann, E. J., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 822.

[6] Webster, B., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1026.

[7] Baumann, E. J., Cipra, A., and Marine, D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1017.

[8] Hoffmann, A. W., *Berichte d. chem. Ges.*, 1874, **7**, 1293.

[9] Hoffmann, A. W., *Berichte d. chem. Ges.*, 1874, **7**, 520.

[10] Hoffmann, A. W., *Berichte d. chem. Ges.*, 1874, **7**, 518.

TABLE I.

Group	Sex	Age (mo.)	Substance	How Given	Dose	Condition of Thyroid		
I	M	4	Methyl cyanide	With rolled oats	0.5 cc.			+
	F	9	" "	" " "	"		+—	
	F	4	" "	" " "	0.25	—+		
	F	9	" "	" " "	"	—?		
II	M	4	" "	Subcutaneously	0.10			++
	M	4	" "	"	"			+
	F	4	" "	"	"	—		
III	M	3	Allyl cyanide	"	0.0125			+?
	F	5	" "	"	"		+—	
IV	M	4	Benzyl cyanide	"	0.025			+?
	M	4	" "	"	0.019		+—	
	F	4	" "	"	0.0125	—+		
V	M	5	Phenyl cyanide	"	0.04	—?		
	M	5	" "	"	0.02	—+?		
	M	5	" "	"	0.01	—?		
VI	M	4	Cyanamide	"	0.20	—+		
	F	4	"	"	0.10	—		
	M	4	"	"	0.05	—		
VII	F	5	Sod. thiocyanate	Intraperitoneally	0.20	—		
	F	6	" "	"	0.10	—		
	M	5	" "	"	0.05	—		
	F	5	" "	"	0.025	—		

++ = More than twice normal size, very hyperemic.
+ = Twice normal size, very hyperemic.
+— = One and one-half normal size, moderately hyperemic.
—+ = Slightly enlarged, slightly hyperemic.
— = Not enlarged, not hyperemic.

The thyroid response was more marked in young rabbits. So far our experiments to prevent these cyanides from causing thyroid hyperplasia by the administration of sodium thiosulphate intraperitoneally have been negative.

Our experiments so far have demonstrated that substances which depress oxygen consumption may increase thyroid activity and that cyanides are among the most potent of these goitrogenic agents. While a cyanide has not yet been definitely isolated from cabbage, enough evidence is available to show that its goitrogenic activity is due to one. These observations have given us our first definite information concerning the essential cause of thyroid hyperplasia. We have frequently pointed out that a deficiency of iodine, while certainly the immediate cause of thyroid hyperplasia, is in most cases only relative, and is due to the increased demands for iodine caused by a goitrogenic agent.

Iodine administration will prevent hyperplasia of the thyroid caused by cyanides just as easily as the hyperplasia due to meat diets (liver), cabbage feeding, administration of anterior pituitary

extracts, or following the partial removal of the gland. These observations throw some light on why a low iodine intake may not lead to thyroid hyperplasia if the production of cyanide is below the effective concentration or if the mechanism for detoxicating cyanides is sufficient, and why a high iodine intake may not protect against thyroid hyperplasia if there is an excessive cyanide intake or formation within the body.

If cyanides prove to be an essential factor in the causation of goiter, both the exogenous and the endogenous sources of cyanide must be investigated, since it is obvious that in most cases the cyanide must be of endogenous origin. This must exist in the organism in an effective concentration either because of insufficient detoxification or through some modification of metabolism whereby cyanide is formed in excess of its physiological needs or of the organism's capacity to handle it.

The fact that the thyroids of a small percentage of rabbits of the same weight, age and breed did not undergo hyperplasia following injection of cyanides is of biological interest, since it suggests, among other things, that some rabbits have a more efficient physiological mechanism for detoxicating cyanides.

The effect on the thyroid of feeding plants known to contain large amounts of HCN *(Sorghum vulgare, Sorghum sudanense)* has not been studied.

6074

Cortical Response to Stimulation of the Optic Nerve.

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In the cortex of the rabbit a small area can occasionally be found that is supplied by one artery and one vein. Such a region can thus be isolated by incisions except for the tongue of tissue where the vessels enter, without serious interference with the blood supply. A metal plate slipped under this tissue and connected to ground serves as an indifferent electrode, and the end of a fine wire resting on the cortical surface serves as a test electrode. When the region so isolated is not active no record is picked up from activity in the rest

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of the cortex. In 2 cases such a preparation has shown activity, and its pathway could be traced via the tongue of intact tissue. The activity consisted of a succession of wavelike action potentials, about 3 per second, lower than those of the adjacent intact cortex and much less complex. The rotating interrupter of the oscillograph apparatus was adjusted to a speed almost synchronous with these waves, but slightly slower, so that successive waves appeared to progress slowly across the screen. If a stimulus is sent in to the tissue at each revolution of the interrupter, it will fall later and later in successive waves. When stimulated during the negative phase or immediately afterward, no response is elicited, the response becoming larger the later the stimulus falls in the cycle. The response consists of a wave but little shorter than the spontaneous wave, and inhibits the following wave, in which case there is a compensatory pause, but it does not otherwise alter the rhythm which is imposed from without the circumscribed region. The response to stimulation is too protracted to be assignable to nerve fibers directly stimulated, and is presumably due to nerve cells.

Upon direct electrical stimulation of the optic nerve, a similar phenomenon can be recorded from the intact optic cortex. The normal activity here is so complex that no simple rhythm can be detected. However, upon repeated stimulation of the nerve at a frequency of one per second, in certain regions of the cortex weak stimuli to the nerve (few fibers responding) give only occasional responses, stronger stimuli permit only occasional failure of response, and still stronger stimuli give a response whose amplitude rises and falls periodically with a slow rhythm. Presumably at some point along the pathway the stimuli coming up from the nerve reach a region undergoing rhythmical activity, and find this region refractory part of the time.

That this critical region is in the cortex itself is indicated by the fact that other points on the optic cortex do not show this rise and fall of response, each stimulus being equally effective.

The response consists of 2 or more discrete waves about 40 sigma in duration and ½ mv. in amplitude or less, depending in part on the stimulus. The first 2 waves in the response are small and are not always seen, on account of the distortion of the record by the stimulus. The succeeding one is much larger and always exists when the system responds to the stimulus, while the following waves vary. The present evidence tends to show that with increase in the strength of stimulus these larger waves come earlier. The response is present over a considerable area of the occipital cortex on

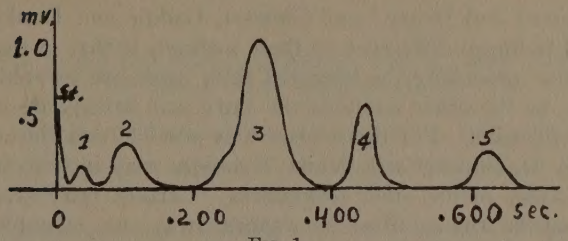

FIG. 1.

Composite analysis of series of records with different strengths of stimulus, showing 5 successive potentials in response to 1 stimulus to optic nerve.

either side and is higher on the crossed side, but is not present in other regions of the cortex. These latter regions are among the places where the spontaneous activity in the cortex is at its highest.

The normal record is presumably too complex to permit detection of the particular rhythmic function responsible for the refractoriness of the optic pathway, even if the proper region were precisely located. We believe, however, that this experiment corresponds in the intact cortex to the previous experiment in an isolated region where the phenomenon could be directly observed. The inference may be drawn from these and other experiments that certain groups of cells in the cortex are rhythmically active, probably spontaneously and automatically; that these foci originate impulses that spread over complex pathways to other regions, several pathways being represented at any one locus where a point electrode leads off a complex record; and that afferent impulses to the cortex may both modify the activity of cells that are already rhythmically active and set quiescent cells into activity.

6075

Note on the Determinations of Blood Fat.

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The technique of Stewart and White[1] for the determination of "blood fat" and its modifications by Himwich, Friedman and Spiers[2] has yielded higher figures than have the methods developed

[1] Stewart and White, *Biochem. J.*, 1925, **19**, 840.

[2] Himwich, Friedman and Spiers, *Biochem. J.*, 1931, **25**, 1839.

by Stoddard and Drury,[3] and Stewart, Gaddie and Dunlop.[4] The essential technical difference of these methods is that in the Stewart and White[1] procedure the liberated fatty acids are titrated directly, whereas, by the other methods the fatty acid precipitate is washed prior to titration. For this reason it is possible that the values obtained by the Stewart and White[1] technique may include substances washed away in the other procedures. Certain fatty acids of the blood may be among those so washed away, for example, arachidonic and other unsaturated fatty acids. On the other hand, extraneous acids may also be lost in the process of washing, such as phosphoric or organic acids.

The quantitative aspects of arachidonic and other unsaturated fatty acids of the blood are to be investigated in this laboratory. Stewart, Gaddie and Dunlop[4] suggested that phosphoric acid liberated from lecithin is the substance responsible for the difference in results obtained by these methods but we have not found it possible to determine any significant increases in inorganic phosphorus as a result of the Stewart and White[1] procedure. On the other hand, the possibility remained that inorganic acids arising from the breakdown of glucose might account for some of the divergencies in results. Therefore, determinations of the "fat content" of the plasma with and without the addition of glucose were made by the method of Stewart and White.[1] It was found that the titration value of "fat" rose when glucose was added to plasma. The effect of reducing substances in the blood, however, is not always identical with that produced by equal quantities of added glucose for the reducing substances yielded a smaller titration than did the added glucose. In view of these newer findings it becomes evident that the data hitherto obtained by these methods may require revision.

[3] Stoddard and Drury, *J. Biol. Chem.*, 1929, **84**, 741.
[4] Stewart, Gaddie and Dunlop, *Biochem. J.*, 1931, **25**, 733.

6076

Protection of the Rat Against Infection with a Larval Tapeworm by Serum from Immune Rats.*

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No successful attempt to inhibit or prevent development of a metazoan parasite by injection of immune serum has been recorded. It has previously been shown that the rat can be artificially immunized against infection by onchospheres of the tapeworm *Taenia taeniaeformis* by injections of worm material[1]; and that rats infected with the larval stage of this worm, *Cysticercus fasciolaris* are thereby protected against superinfection.[2] Serum from artificially immunized rats was later found to confer a high degree of protection against infection by onchospheres of this cestode.[3] The results of 2 experiments show that complete protection has now been secured by the injection of serum from rats infected with cysticerci.

In the first experiment 36 rats from 4 litters were evenly divided into 3 groups of 12 each. All animals were given equal portions of a uniform suspension of onchospheres by stomach tube. The 12 rats of group C were reserved for controls; 2 hours after feeding onchospheres the animals of group A received pooled serum from infected rats, and those of group B serum from artificially immunized rats. In the 2 latter cases 1 cc. of serum per 25 gm. of body weight was injected intraperitoneally. All rats were autopsied 37 days later; summarized data are shown in Table I.

Similar results were obtained in Experiment 2, in which 31 rats

TABLE I.

12 rats in each group. Figures give the average number of cysts in liver.

Group	Injected with	Living (2 to 6 mm. diam.)	Small dead
A	Serum from infected rats	none	none
B	Serum from artificially immunized rats	0.5	6.0
C	Controls	22.2	18.7

* These investigations were in large part carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Miller, H. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 926; *J. Prev. Med.*, 1931, **5**, 429.

[2] Miller, H. M., Jr. PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 467; *J. Prev. Med.*, 1931, **5**, 453.

[3] Miller, H. M., Jr., and Gardiner, M. L., *Science*, 1932, **75**, 270.

from 4 litters were used. Sixteen control animals received injections of serum from uninfected rats, and 15 experimental animals received pooled sera from infected rats. One cc. of serum was given per 25 gm. of body weight before, and again immediately after feeding onchospheres. All animals were autopsied 32 days later; the data are summarized in Table II.

TABLE II.

Figures give the average number of cysts in liver.

No. of Rats	Injected with	Living (2 to 6 mm. diam.)	Small dead
15	Serum from infected rats	none	none
16	Serum from uninfected rats	52	1.5

Further experiments are in progress to determine the minimum amount of immune serum necessary, and the duration of the protection conferred by such serum.